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A fissure eruption in November 1978 on the Asal graben, southernmost spreading segment in Afar. A 40 m high spatter rampart was erected on the fissure and 16 m^3 of basalt was extruded. In the background are the normal faults of the eastern scarp of the graben. For an examination of these seismic and volcanic events see p.17. New isotopic data are presented on p.56.

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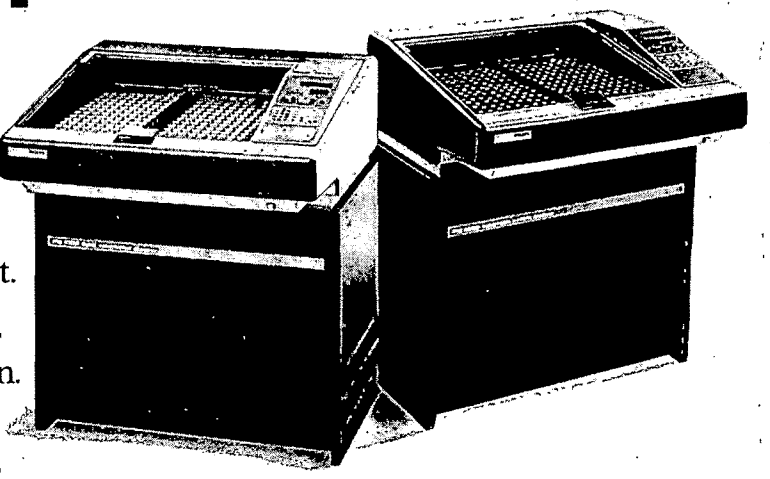
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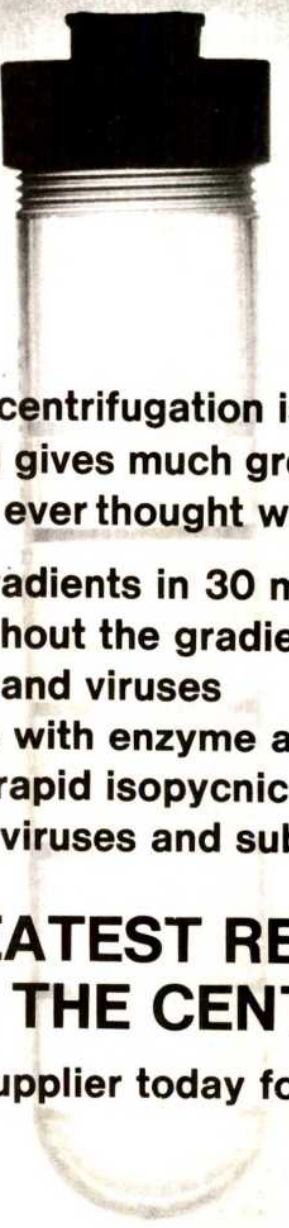
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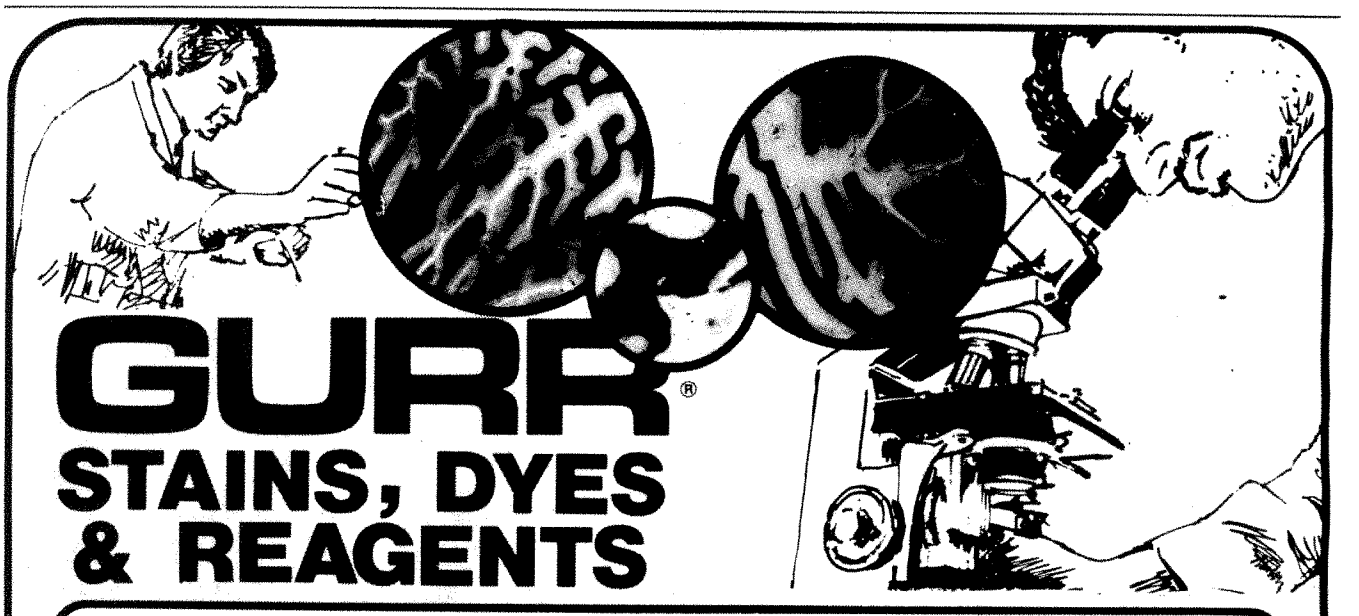
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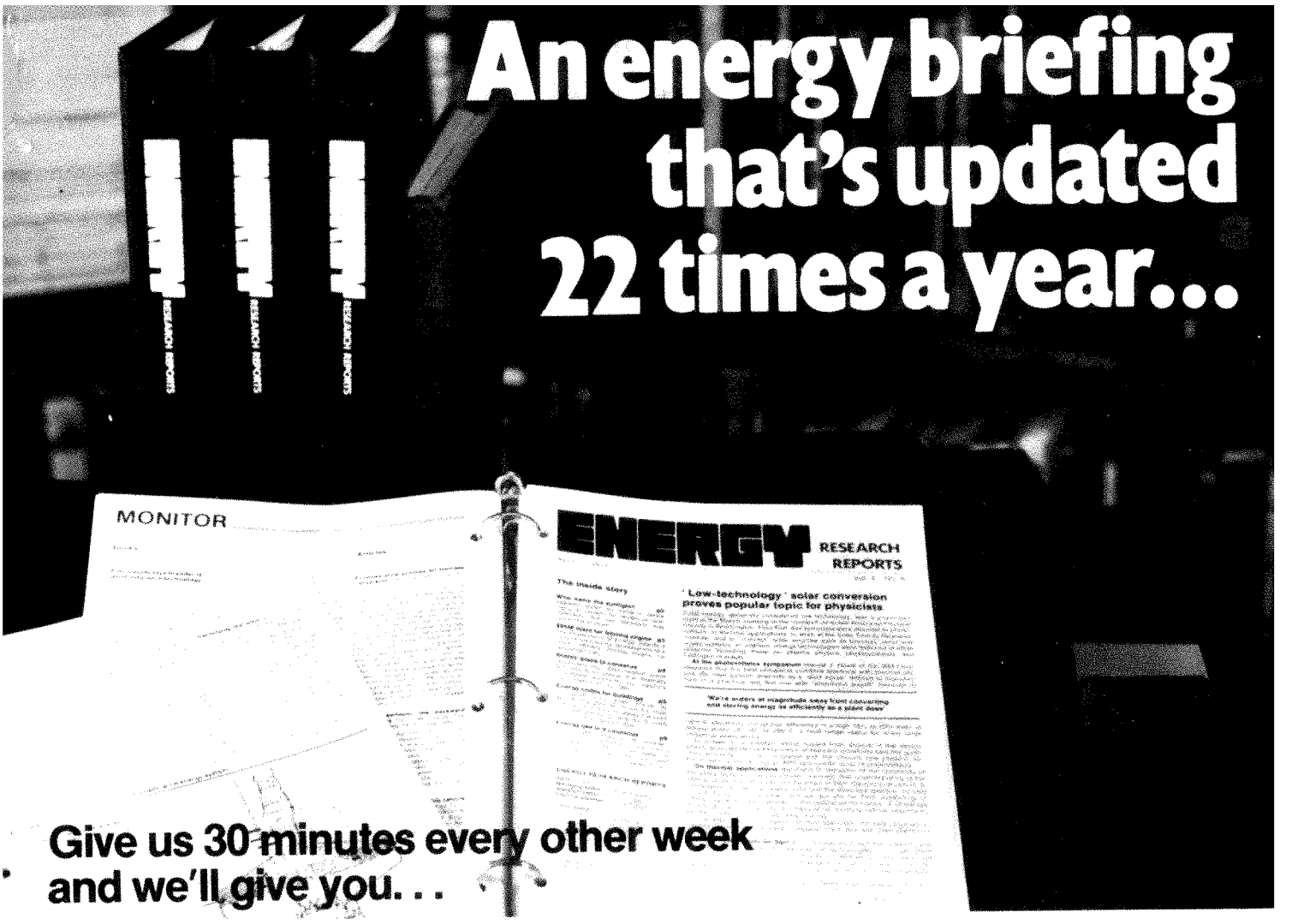
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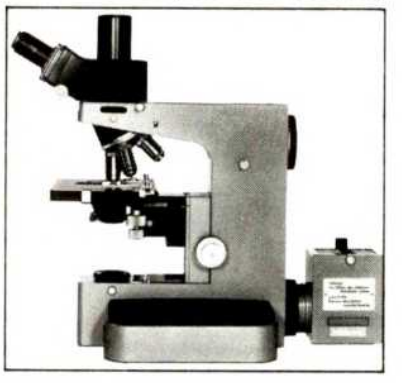
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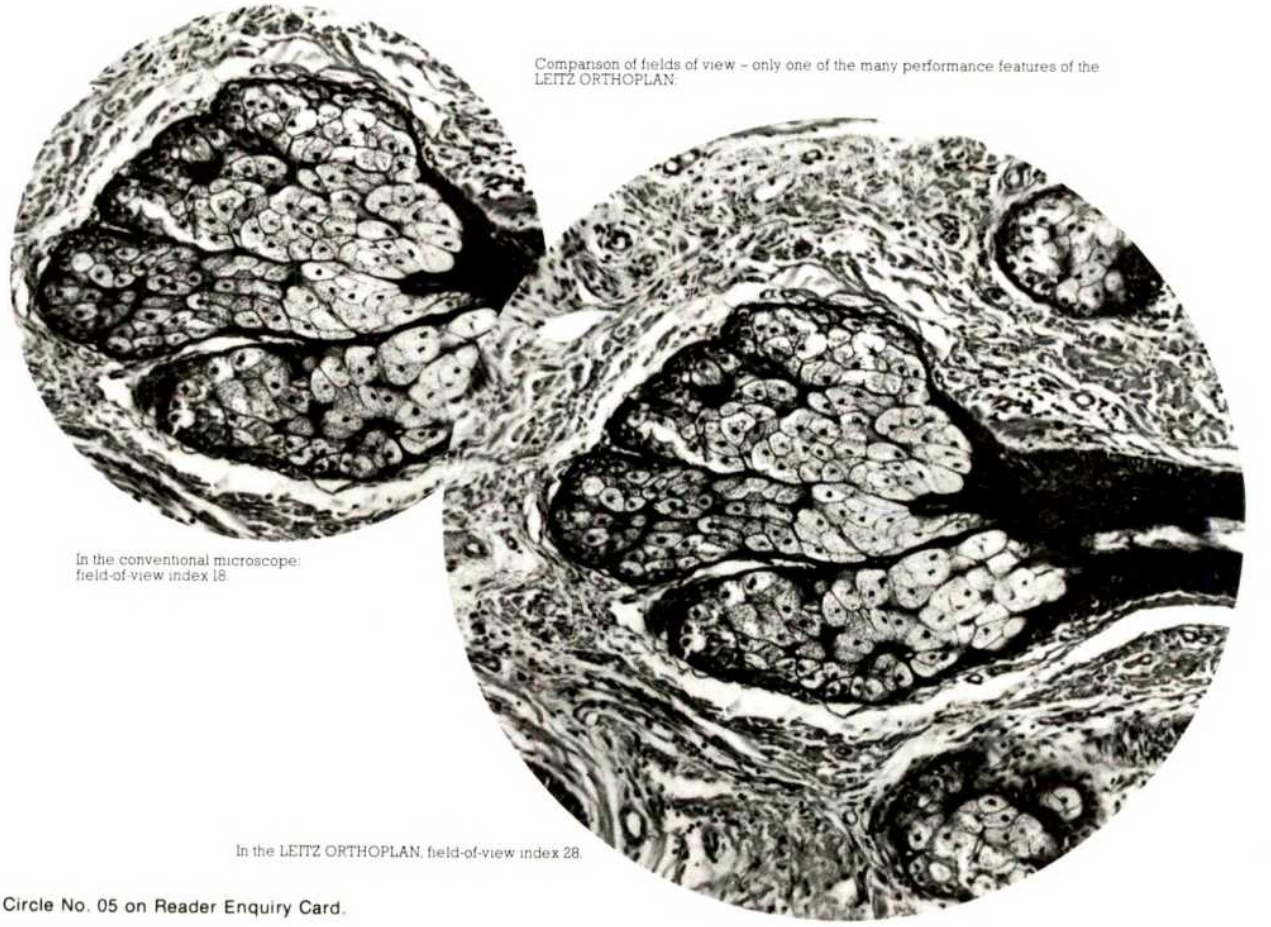
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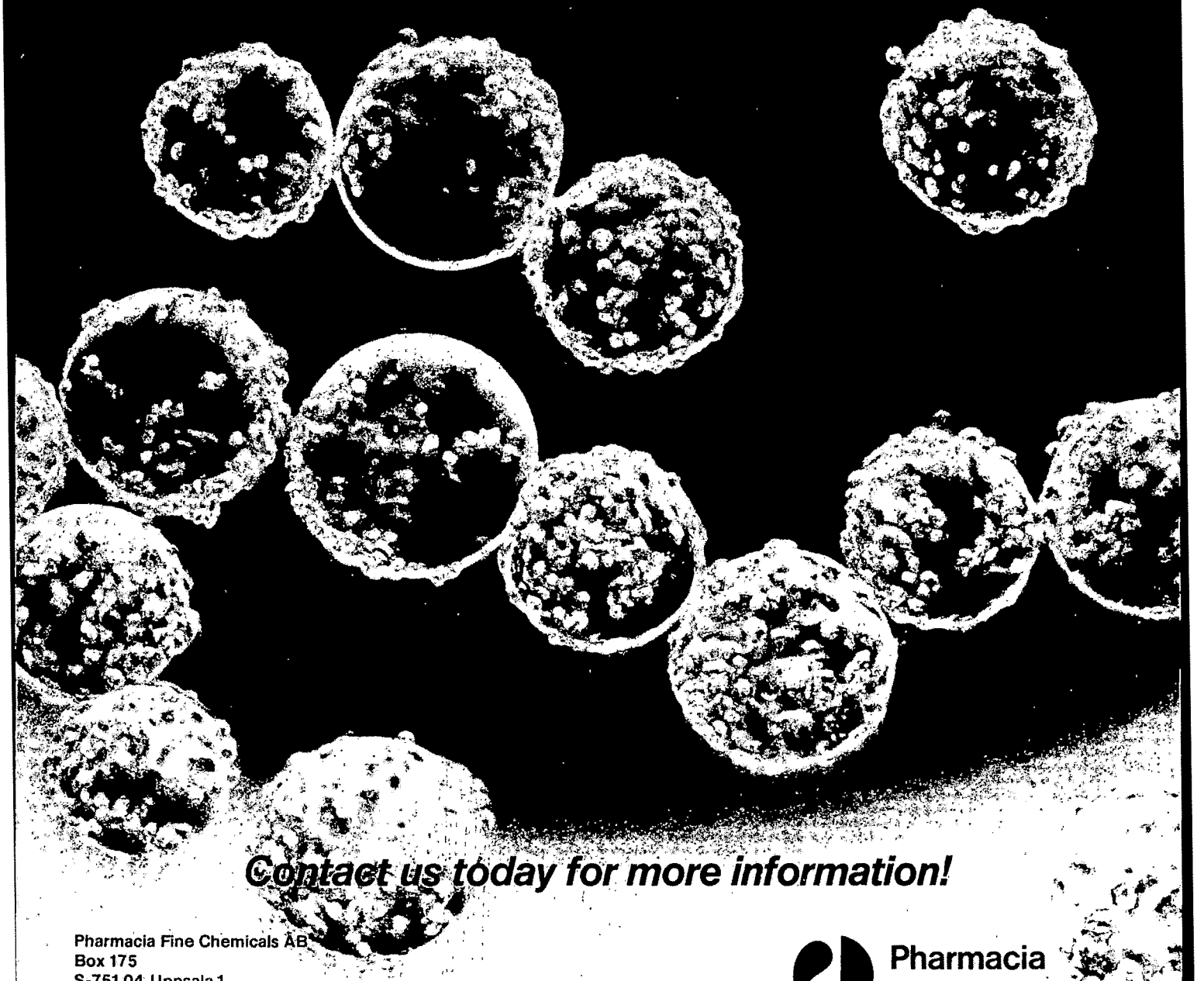
Erratum

The paper 'Local destabilisation of a DNA double helix by a T-T wobble pair', Nature **281**, 235 (September 20th), was printed with errors in the author line. It should read: A.G. Cornelis Haasnoot, Jeroen H.J. den Hartog, Jan F.M. de Rooij, Jacques H. van Boom & Cornelis Altona.

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1 November 1979

Time for a policy on scientists' jobs

SHIRLEY Williams, when she was Secretary of State for Education and Science, was apt to complain that there was no vocal lobby for science, although there was for everything else from aardvarks to zither players. Well, that is about to be rectified when next Tuesday representatives of the Association for Researchers in Medical Science (ARMS) lobby the House of Commons about increasing unemployment among scientists.

Anne Simmonds, the convenor of ARMS, in an interview this week (p7) explains that she herself never expected to be redundant when she set up the organisation; she is a widely respected biochemist whose case underlines the difficulties and inconsistencies of assuming that in a time of cuts the "truly able" (in the words of the Medical Research Council) will find appropriate scientific employment. Moreover, it is misleading for the MRC to claim, as one official told *Nature*, that short term contract workers "are not geniuses". While this is no doubt the case (for most), it draws attention from another unpalatable but undeniable fact that many of the tenured positions filled during the boom years of the late fifties and early sixties are occupied by people less able than many in the queue behind them.

But this is merely to pose the problem: a policy of the "truly able" is one which fudges the figures to match the facts; the number of "truly able" is by definition the number who get jobs; the argument is circular and it is no policy at all.

What is needed, and urgently, is some hard thinking about the nature of scientific careers and what government can do to make proper use of this absolutely central innovative resource. It is utter folly for a government bent on establishing economic recovery to waste its scientific resources — its scientists — through a *laissez faire* policy which both wrecks individual careers and discourages those rising on the ladder. The Science Research Council, which is responsible for most basic research in the UK, has some forward looking policies, particularly on the relation of science to industry, which must be encouraged; and the Medical Research Council which appears to be relatively backward in this regard would do well to follow suit. But some central government initiatives are required to coordinate these efforts within a broad demographic policy for science and engineering. Here is a case where science is too important to be left to the scientists, a case where the Prime Minister should exercise her stated aim of coordinating science policy where that is necessary (see *Nature* 27 September p249).

One particular matter to which she might pay attention is the role of research students, which is the engine of production of the "excess" of short-term contract workers. The pattern of using these students as a form of cheap labour for adding to a researcher's list of publications arose during the expansion of the universities, when it was virtually guaranteed that this apprenticeship would lead to a university position. This pattern continues, although the job at the end is no longer there. Breaking the pattern by greater selectivity in appointments to studentships (perhaps by examination) carries with it the further problem of what substitute technical assistance can be provided for existing researchers, and at what cost; and how to place those graduates who would at present go on to research. But this would bring the 'jobs problem' in a scientific career back to the age of 21, where it is considerably more tractable than at 24 after a PhD, or even 30 and beyond after a series of short-term contracts. We recommend to you, Prime Minister, that you ponder these matters, to the benefit not only of science, but also of the British economy. □

Soviet germ disaster shock?

SPECULATIONS on possible Soviet research into bacteriological and chemical weapons were revived this week by *Now!*, Britain's recently-founded answer to *Newsweek*. A two-page article, entitled "The Great Russian Germ War Disaster", gives a circumstantial account of an accident at a factory in Novosibirsk, last June, shortly after which, persons living in the vicinity "started to go down with a mysterious illness". "Thousands" of people, it is said, were affected, with the death rate among the victims "very high". The bodies of those who succumbed, it is reported, were delivered to the relatives in "sealed coffins". When a few relatives succeeded in examining the bodies, they were found to be covered in "brown patches".

The story, *Now!* makes clear, was nowhere reported in the Soviet press, but was transmitted to *Now!* by a "traveller". It should be recalled, however, that except for international air-crashes or accidents involving foreigners, it is very rare for the Soviet press to report accidents. Certainly, to judge from the *Now!* reports, something untoward happened in the Novosibirsk factory — but the account, as *Now!* itself admits, is consonant with a Séveso-type chemical disaster, which need not necessarily have any connection with the military at all. The allegation of bacteriological warfare seems to be based on the fact that a molecular biology institute was established at Novosibirsk some three years ago.

Certainly, the now famous Kyshtym nuclear devastation of 1958 is a constant reminder that the Soviet Union has the logistic capacity to mount a major cover-up operation, even to the extent of evacuating large tracts of country. Moreover, in conditions of a controlled press, even the most innocent natural disaster is liable to generate wild rumours. (Last winter's explosion, which wrecked the Rotunda building in Warsaw, was attributed, in popular belief, to a bomb planted by the authorities . . . so that the dissident "Committee for Social Self-Defence" issued an appeal to the government to allay panic by publishing the result of the commissions of enquiry).

The official Soviet News Agency TASS always vehemently denies any rumours of bacteriological weapons research. Nevertheless, if what happened at Novosibirsk had no military applications, it is perhaps unfortunate that, immediately following the *Now!* allegations, the Soviet press attache in London was away on sick leave, leaving no deputy empowered to comment. □

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Can Yankee ingenuity keep US physics on the ball?

As US high energy physicists bemoan a continued tightness of funds, West Coast scientists are proposing an ingenious way of keeping up with European colleagues — at a fraction of the cost. **David Dickson** reports

Inflation is threatening to upset the fragile truce agreed two years ago between the US research community and the Office of Management and Budget over the appropriate level of support for high energy physics.

The agreement negotiated by the Department of Energy guarantees a constant 'floor' of support (adjusted for inflation) for research at the three main accelerator laboratories — the Fermi National Laboratory, the Brookhaven National Laboratory, and the Stanford Linear Accelerator Center — pegged at the 1979 level of $300 million a year; but it promises no more, unless a particularly good case can be argued.

Initially physicists accepted the agreement since although it means less money than they would have liked, at least it provided medium-term security. But now a higher-than-anticipated inflation rate means that the physics budget is falling considerably behind steady-state funding, perhaps by $30 million over the next two years; and this is having serious implications for current and projected research programmes.

It also increases uncertainty over whether Congress can be persuaded to support two projects which would enable US physicists to keep up with their European counterparts as the latter pursue the intermediate vector boson (Z^0) predicted by the Nobel-prize-winning theories of Steven Weinberg, Abdus Salam and Sheldon Glashow.

The first is a new facility proposed by Fermilab to help investigate the proton-antiproton collisions that should produce the Z^0; the second is an ingenious plan devised by Dr Burton Richter of SLAC to study the physics of the Z^0 and its decay particles at a fraction of the cost of Europe's proposed 100 GeV electron-positron collider, LEP.

Compared to the early 1970s, the current picture for US high energy physics is far from gloomy. Indeed from one point of view the community is doing very well, with major construction projects under way at the three main laboratories, as well as Cornell University. "The current programme is basically sound, and there has certainly been a reversal in prospects since 1975" says Dr Tony Samios of Brookhaven.

There are, admittedly, some slight hitches. At Brookhaven, Long Island, for example, designers working on ISABELLE, the 400 GeV intersecting proton storage rings, are having difficulty in getting the superconducting magnets to produce the required magnetic field; it's a problem which has led to "particular agony over the past few months", says project director Dr Jim Sanford, though he remains confident that it can be solved by the time the magnets should be ready for mass production next spring.

Elsewhere the picture is brighter. Fermilab near Chicago claims to be up to schedule with its own production of superconducting magnets, designed to double the accelerator's energy from 500 to 1000 GeV; SLAC, in California, injected its first positron beam into its positron-electron collider PEP last week-end, and is planning to start operating on schedule — and within the $78 million budget — in late January 1980.

The biggest cloud around is rising inflation. The DoE's budget allocation for the current fiscal year allows for 7% inflation. In real terms, inflation in the US is well into double figures; and construction costs on new facilities have increased even more. The projected 1981 budget of around $350 million could be about $20 million less in real terms than the 1979 level previously promised.

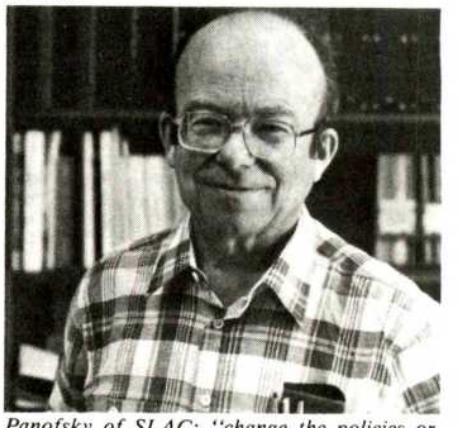
Of particular concern are the high electricity costs caused by oil price increases, a factor to which accelerators, with their vast power consumption, are particularly vulnerable. Brookhaven, for example, has recently been faced with an almost 50% increase in its power rates; and at SLAC, the extra power needed by PEP will require shifting to a new source of supply at much less preferential rates, which will increase the laboratory's annual power bill from $2 million to almost $5 million when PEP is fully operational.

Since salaries are fixed, the extra costs will have to be absorbed by operating budgets. The result could be a significant curtailment of experiments. SLAC is already warning that when PEP comes on line, it will have to operate considerably below capacity; and Dr Leon Ledermann, Fermilab director, is talking of having to close down a complete experimental facility, the 15-foot bubble chamber used for neutrino-beam experiments, if additional support is not forthcoming.

Up to now, physicists have told the DoE they were confident a viable national programme could be maintained at the three accelerators on the $300 million figure. "Now either the policies have to be changed or the funding has to be changed; we cannot pay lip service to both", says Dr Wolfgang Panofsky, director of SLAC.



Ledermann of Fermilab: experiments may have to close



Panofsky of SLAC: "change the policies or change the funding"

What makes the situation particularly frustrating is that US physicists see themselves having to sit back while their European colleagues using new facilities such as the super proton-synchroton (SPS) at the European Centre for Nuclear Research (CERN) and the proposed LEP, explore the new areas of physics that will be opened up if the existence of the Z^0 particle is confirmed.

The two proposals now on the drawing board for keeping up with these developments would therefore fill a major gap that could develop within the US high energy physics programme. Fermilab's plan is to build a new machine which will help physicists to study the proton-antiproton collisions expected to produce the Z^0.

The plan requires a pre-booster storage ring, which would collect anti-protons created by protons extracted from Fermilab's main ring, and cool them sufficiently to reinject them in the opposite direction to the protons. The machine would come on line after PEP, which is expected to be the first to discover the Z^0 (if

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it exists) soon after coming into operation in 1981 (perhaps). It would produce a considerably higher collision rate than the equivalent European machine; and Fermilab physicists are hoping that this will help persuade Congress to provide the necessary $26 to $29 million.

The second proposal, devised by Dr Richter, is to create single collisions between individual pulses of electrons and positrons. Both would be produced on the SLAC linear accelerator, using techniques now being developed to increase the beam energy from around 25 GeV to 50 GeV; they would then be made to circulate in opposite directions around a circular tunnel, and carefully aimed to collide with each other.

Since each pulse will only produce a single collision, the event rate will be much lower than for the constantly circulating pulses in storage rings at a comparable energy. Dr Richter proposes to make up for this, however, by tuning the two beams to collide at a centre of mass energy calculated to be equal to be the mass of the Z^0 particle, using the resonance at this energy to increase the luminosity.

If the Z^0 does exist, then the Single Pass Collider, as the project is known, would become a virtual factory of decay particles with an event-rate around 10^6 a year, and the facility could be used by many different users. If the Z^0 does not exist, the event rate will be very much slower; "but the physics will be much more interesting, since it would mean a basic flaw in the theory", says Dr Richter.

The design would lack the power and versatility of LEP, scheduled to reach 100 GeV per beam, and to be built by the late 1980s. But at a cost of $60 million — compared to LEP's $1000 million — plus the fact that it could be in operation as early as 1984, the proposal is attractive to US physicists. "It is an extremely clever idea, real 'Yankee ingenuity' " says one.

It all comes down to money. Dr Richter has asked the DoE for $800,000 to carry out a preliminary design study; and says that, if given the go-ahead by the department and Congress in the 1982 fiscal year budget to be prepared next summer, the collider could be completed in under three years.

Whether Congress will go along with either this or the Fermilab project remains the great unknown; and few are over-optimistic. Last week the DoE's High Energy Physics Advisory Panel (HEPAP) supported a proposal that its chairman, Dr Sidney Drell of SLAC, write a letter to the department expressing grave concern at the current funding position; and warning that if there was not an immediate infusion of extra funds — at least sufficient to restore the budget to the promised "floor" — the panel would consider withdrawing its endorsement of the national programme. Department officials at the meeting promised the physicists a sympathetic hearing; but no more than that. □

NIH director unlikely to grant exemption from controls for DNA experiments

The director of the US National Institutes of Health, Dr Donald Fredrickson, seems unlikely to accept in full the recommendation of the Recombinant DNA Advisory Committee (RAC) that a large body of recombinant DNA experiments be exempted from the guidelines established by the NIH, following widespread criticism of the implications of such a move.

Sources within the NIH say that Dr Fredrickson will probably approve a significant reduction in the safety precautions needed to carry out most experiments using as host the disabled K-12 strain of the bacterium *Escherichia coli*; these are said to account for between 80 and 85% of all experiments using recombinant DNA techniques.

But whereas the RAC, in a split 10 to 4 vote, decided at its last meeting in September to recommend *exemption* for the guidelines for such experiments — with merely the ruling that they should be registered with local biohazard committees, and should preferably be carried out at the minimal P1 physical containment level — Dr Fredrickson is likely to insist that the P1 conditions be enforced. This will include strict adherence to technician training requirements, and a ban on practices such as mouth-pipetting.

The NIH has had a flood of comments since the proposal to exempt the experiments was first put forward by RAC members Dr Allen Campbell and Dr Wallace Rowe at the committee's meeting in May. Many scientists wrote supporting the proposal, including in particular a petition signed by 183 scientists attending the Gordon conferences on nucleic acids and on biological regulatory mechanisms.

Others, however, have expressed concern about the implications of such a drastic move. Professor Roy Curtiss, of the department of microbiology at the University of Alabama in Birmingham, wrote to Dr Fredrickson criticising the proposed exemptions as premature, given the uncertainties that still exist over the hazards of recombinant organisms. "I believe that the RAC's recommendation to you was based more on the politics of science than on its data", he wrote.



Fredrickson: reviewing the arguments

In addition, the Natural Resources Defense Council in New York has demanded that an environmental impact statement be carried out for the proposed safeguard reductions, arguing that "the proposed exemptions are of such breadth and importance as to require full compliance with the National Environmental Policy Act."

In view of the controversy surrounding the committee's recommendation and its implications, Dr Frederickson has been carrying out a detailed review both of the arguments used during the RAC meeting, and of the data used to support them.

The results of this review will not be known for several weeks. However it is thought that Dr Fredrickson is reluctant to face the criticism that total exemption would involve.

For example, two weeks ago the citizens of Amherst, which has introduced a local ordinance requiring recombinant DNA research at the University of Amherst to be conducted under the NIH guidelines, agreed to require adherence to the 1978 revision of the guidelines, rather than their stricter 1976 original version.

However according to Dr Bruce Levin, a research scientist at the university who has been closely involved in the local debates, there would probably be strong resistance from the local community if a large proportion of the experiments were to be exempt from regulation.

Dr Fredrickson, however, is said to be prepared to accept most of the arguments in favour of a substantial reduction in required containment levels for many experiments involving the K-12 strain; one of the few areas still to be resolved is whether local committees would be required to give prior approval to experiments using biologically active materials, such as active polypeptides or active proteins.

The NIH is paying particular attention in its review to uncertainties that have arisen as a result of various risk assessment experiments, such as those which indicate that bacteria into which plasmids have been introduced can survive considerably longer than expected in the human gut.

Meanwhile staff members for Senator Adlai Stevenson's science and technology subcommittee are preparing legislation that would require all non-federally supported research involving recombinant DNA techniques — in particular that carried out by private companies — to register their experiments with the NIH. At present companies can register; but the arrangement is voluntary. □

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Pollution control: how an EEC directive muddied the waters

Britain and the EEC have been arguing for many years about the best way of controlling pollution. The conflict arose not because anyone disagreed that certain substances should be controlled but because the EEC wanted to lay down a blanket standard of emission into rivers from sewers and factories.

Britain argued that this was wasteful and inefficient because it took no account of the use of a particular river nor its capacity to dilute and disperse waste materials. Britain wanted local controls, based on local conditions and local usage, as long as there was no risk to human health or animal life.

Eventually, in December 1975, the EEC Council of Ministers compromised and agreed to let the British control pollution in their own way. But now new directives have been issued from Brussels concerning the control of specific substances in water and they put a question mark over that 1975 agreement



Extracts from a speech by Lord Ashby (right) to the House of Lords in London last week

The first of the 'drin' Directives sets a limit for the emission of dieldrin. It is 60 parts per billion in the waste water discharged into the river. Clearly, the Commission considers that this is going to be a little dificult because it allows factories time to build treatment plant. The limit is not to become binding until 1986, six years ahead. The second Directive offers the other option, the one we asked for: an environmental quality objective for the river into which the wastes are discharged. The level proposed for this is 0.005 parts per billion. Unlike the first option, this level has to be reached in two years' time, which gives the impression that it is an easier option.

These requirements make a nonsense of the options agreed by the Council of Ministers after great pressure from the British Government in 1975. First, the two options are mutually contradictory and inconsistent. Suppose we complied with the first Directive. Suppose we reduced emissions to 60 parts per billion in the Calder River (the river most affected in the UK). Would this result in an environmental quality objective of 0.005 parts per billion? It certainly would not. After doing a little arithmetic, which is fully within the capacity of the staff of the Commission, it has been found that it would need a flow of one hundred times that of the Calder at its minimum value in order to produce that degree of dilution; in fact many times the flow of the Thames at Teddington.

So the two proposed standards simply do not match. This could either be a deliberate soft option for those member states that want to choose an emission standard or it is a sign that the scientific basis for these figures is very suspect. That is not the only difficulty. The standard of 0.005 parts per billion is inconsistent with two other Directives which have already been published by the Commission and agreed by the Council on their own definition of pollution. For instance, the Commission has already agreed in principle that the level of dieldrin in water to be used for human consumption is to be not more than 0.1 part per billion — that is, 20 times higher than the level proposed in this Directive — so the limit has obviously not been set to make the water safe to drink.

What about the fish? Three years ago the Commission published its own water standards for fish. In this Directive, which has now been adopted, pesticides are not included at all. However, it is possible to calculate what the safe level will be because it is known that for a coarse fish like the roach — and we are talking about coarse river fishery in the Calder — about two parts per billion is the beginning threshold value for damage, and the level of dieldrin in the Calder varies a good deal; but it is in the region of 0.1 part per billion, that is one-twentieth of the danger level.

So on two counts, both falling in the definition of pollution by the Commission itself — human health and the health of fish — the environment quality objective proposed just does not make sense. There remains a very important fact, that the fish and other living things accumulate dieldrin from water, and they concentrate it enormously in their own tissues. If people then eat the fish they might be at danger. Experts are reluctant to commit themselves to precise figures for the amount of dieldrin which fish and other living things will accumulate from the environment. However, it is a general consensus that it is a multiple of 10,000 of the concentrations outside.

If the Calder carried trout, salmon or eels — which it does not because of pollutants other than dieldrin — then there would be an indubitable case for monitoring the dieldrin level of the fish. Then consent levels on the river could be imposed which would guarantee that the acceptable daily intake, if people ate the fish every day would be safe. This is exactly what is being done all the time in Britain in waters where fish are eaten. But coarse fish are not eaten in Britain, and the present use to which that stretch of water is put does not justify such drastic lowering of the dieldrin level either — to come back to the Commission's own definition — on grounds of hazards to human health or on grounds of harm to living resources.

Finally, there is a practical difficulty about settling a standard of 0.005 parts per billion which the drafters of the Directive do not seem to have noted. The waters which fall into a river from a mothproofing plant such as those on the river Calder (these plants produce the dieldrin) contain many impurities. If one wants to analyse the water containing those impurities for the concentrations of dieldrin, one simply cannot detect levels lower than about 0.01 parts per billion, so the level proposed in the Directive is one which could not in fact be measured, except by most refined and difficult research techniques. So the idea of monitoring concentrations of that level as a routine matter simply is not on.

These are the reason why I think it is not an exaggeration to say that "nonsense" is the word that has to be used for this option offered to the nation states in the Community which prefer to take environmental quality objectives rather than emission standards. How did the Commission come across this figure? The Directive states that the figure was reached "after consulting a group of national experts", and also on the basis of a study made by consultants in the Agricultural University of Wageningen in Holland. But as with previous Directives about the environment, scientific evidence for the decision is not given and the consultants' report is not published.

However, I have talked with the British experts in this matter and their view is clear from what I have already explained to your Lordships. I have managed to see a copy of the consultants' report. Again for the record, I should like to read the conclusion of that report: "This leads to the rather disappointing conclusion that most toxicological studies made so far with the aim to assess the environmental hazards of aldrin, dieldrin and endrin . . . have been inadequate in the sense that for the aquatic environment no practical environmental threshold levels can be derived from thier results".

The Commission then concludes, based on that conclusion, without providing any of the consultants' data, that the

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environmental quality should be set at a level which is inconsistent with two other Commission Directives, unjustified on grounds of safety to human beings or wildlife, and below the limits detectable by routine analyses. As one of our witnesses put it, data appeared to have been plucked out of the air using a concentration factor much higher than the one thought to be reasonable by British experts.

We all want to reach amicable agreement with the Commission over an environmental policy for the Community, and we all agree that dieldrin is a harmful substance which ought be phased out as soon as satisfactory alternatives are found. But we see no prospect of amicable agreement unless the Commission can be persuaded to adopt two simple principles when it prepares its Directives.

●The first principle is to abide by a declaration of the Council of Ministers made in 1973 about the environment. This declaration is the only authority for having a European environmental policy at all. It states that protection of the environment should be achieved "at the lowest cost to the Community" and that in setting quality objectives "proper account must be taken of the specific characteristics of the regions in question".

This means the deployment of our limited resources in places where they could be most cost-effective. It means that we should have different environmental qualities for rivers, depending on whether they carry game fish, coarse fish or no fish at all. Following this policy in Britain over the last 20 years, we have increased the amount of unpolluted rivers by about 4,500 kilometres and we have diminished the amount of badly polluted rivers by about 1,500 kilometres. I doubt whether any other member state in the Community can match that record. A doctrinaire pursuit of uniform emission standards would wreck this policy.

●The second principle is to insist on free and candid disclosure of the scientific evidence on which Directives are based. It simply is not good enough for the Commission to say, "After consulting a group of experts, we set such and such standards". The Select Committee has now got plenty of evidence that the experts' advice is not always taken. To comply with some of these Directives — for instance, that proposed for fish — would put member states to enormous expense and to expect them to incur this enormous expense without even seeing the data on which the standards are based is, I suggest, an intolerable situation. I am afraid it is true — and we regret it — that there will be perpetual wrangling over environmental policy in the Community until this freedom of information is secured.

Compared with the Common Agricultural Policy, the Commission's environmental policy for Europe may well be a small matter. But it is a perpetual source of irritation. ❜

UK to tighten asbestos controls

TIGHTER safety standards for asbestos are proposed in a report published last week by the UK Health and Safety Commission (HSC). The report, prepared by the commission's Advisory Commission of Asbestos (ACA) makes proposals, which according to the committee's chairman Mr Bill Simpson would, if they became law, "mean that Britain would share with Sweden the most stringent asbestos rules of any country".

The report recommends that the standards currently in operation regulating asbestos fibre concentration should be halved to 1 fb ml^{-1} for chrysotile (white asbestos); reduced four-fold to 0.5 fb ml^{-1} for amosite (brown); and that a statutory ban be placed on crocidolite (blue) in the UK. In addition, until such fibres as crocidolite are replaced, the standard for its removal should remain at the present 0.2 fb ml^{-1}.

It is proposed that the new standards should take effect from 1 December 1980 and that in future they should be referred to as "control limits" rather than "hygiene standards", the latter definition suggesting an asbestos concentration below which exposure is safe.

Simpson firmly believes that the new standards will reduce the incidents of asbestos-related diseases in future. It is the report's view that exposure to one fibre per mille of chrysotile daily over a period of 50 years will cause an increased mortality rate of 0.02 to 1.25 per cent. This prediction is based on the results of the only three surveys — two North American, one British — which provided adequate dose/response data. Calculations of mortality rates based on these results have their problems, however, as the report readily admits. Much of the data in these surveys on asbestos concentration was obtained by measuring particle counts, a far less precise method than the normal practice of counting fibres.

As far as the public is concerned, the report concludes that there is no quantitative evidence of risk from over-exposure to asbestos dust. The report says that despite the fact that there is a high proportion of individuals, especially in urban areas, with asbestos fibres in their lungs, there has been no general reporting of asbestosis. Accordingly the report suggests that "there may be a threshold level" below which asbestosis is not detectable.

This, however, does not hold true for the carcinogenic properties of asbestos. The report states baldly that there is "no evidence" for the existence of a threshold below which lung cancer or mesothelioma — of the pleura or the peritoneum — will not occur. It is the report's view, however, that crocidolite is consistantly more dangerous than chrysotile and should be treated accordingly. Swedish and Dutch regulatory authorities agree.

The report acknowledges that all forms of asbestos will cause lung cancer. In numerical terms, lung cancer is the great killer but the report says "included in this excess mortality from lung cancer is a substantial element due to the syenergistic action of tobacco with asbestos, which cannot therefore properly be attibuted to the effect of asbestos alone". The asbestos industry has always made much of this synergism to play down the role of asbestos in the aetiology of lung cancer.

On the quesion of death due to mesothelioma, the report is unequivocal; the majority of these are due to exposure by chrysotile. Death from mesothelioma reported in American asbestos workers (the report says) are attibutable to exposure to both amosite and chrysotile and not to chrysotile alone.

Deaths from mesothelioma in the UK doubled between 1967 and 1976 — currently standing at some 320 per annum — and the report expects the number to increase before the peak is reached.

The asbestos industry makes no pretence of being pleased about the December 1980 deadline for enforcing the 0.5 fibre per mille limit for amosite. Mr S D Hardie of Turner and Newall and an advisory committee member says that the deadline is too tight. He insists that working to 0.5 fibres per mille is pushing the technology to its limit. The industry, he says, can work to one fibre per mille of chrysotile but, as far as the 0.5 fibre per mille for amosite is concerned, Hardie says he could not "put his hand on his heart and say to the work force that it is not being exceeded".

Criticism that the advisory committee has been too sensitive to the needs of industry has been voiced by the Society for the Prevention of Asbestos and Industrial Diseases. Mr Max Madden, a trustee of the society and former Labour MP for Sowerby — a constituency which includes the ACRE Mill asbestos factory at Hebden Bridge — was frank about the pressure on the advisory committee. In his view, the committee had been subjected to considerable propaganda from the industry and some of it had succeeded. Anticipating criticism of its report, ACA chairman Bill Simpson emphasised that the control limits are not a "once and for all" exercise. New evidence will be constantly reviewed, and every encouragement was being given to the development of safe asbestos substitutes.

Comments on the report are invited and should be submitted by 31 January 1980.

Alastair Hay

Asbestos Volume 1, final report of the Advisory Committee, £5 (p + p).
Volume 2, papers prepared by the Advisory Committee £5 (p + p) available from HMSO.

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$40 billion law suit over Dioxin damage: A veteran of the Vietnam War is suing five US chemical companies for $40 billion damages, claiming that he suffered from brain cancer as a result of exposure to the defoliant 2, 4, 5-T, known as Agent Orange. The defoliant was used extensively by the US in Vietnam between 1962 and 1971, and recent studies have indicated that its use as a domestic herbicide may be associated with foetal defects in pregnant women. According to the man's attorney, the purpose of the suit is "to establish a trust fund to help the veterans who discover they have been poisoned after the statute of limitation runs out, and for the children yet unborn who will have birth defects".

Waste disposal problems halt radioactive research: Research workers at Harvard University have been told that research involving the use of radioactive tracers will have to be stopped because of difficulties in the disposal of radioactive waste. This follows the closure last week of the US's two main dumping sites for liquid low-level radioactive waste, Hanford in Washington state, and Beatty in Nevada. A third dumping site, Barnwell in South Carolina, refused to accept such wastes last spring.

Many major laboratories and hospitals are now faced with mounting accumulations of liquid waste which will have to be stored until alternative disposal arrangements can be made. The government is considering creating new temporary disposal sites or using the six national laboratories operated by the Department of Energy as temporary dumps. Another alternative could be the incineration or evaporation of the waste products. NIH disposes of its absorbed wastes in this fashion under an agreement with Todd Shipyards in Galveston, Texas. According to Dr Mortimer Litt of Harvard, an insignificant amount of radiation is released by this process, well below the exposure standards established by the Nuclear Regulatory Commission, and would not constitute a public health risk. — *Anne Norman.*

Israeli drought threatens Dead Sea: Israel faces water rationing by next summer, unless there is an exceptionally wet winter to replenish the Sea of Galilee — the main reservoir of the country — now at an all-time low. This year's severe drought will not only have a severe effect on the country's agriculture and industry, it has also caused severe damage to the ecology of the Dead Sea, which gets 65% of its water from the Sea of Galilee via the lower Jordan. Several Israeli scientists are demanding urgent government action on the long-discussed proposal to construct a canal from the Mediterranean to the Dead Sea. Dr Sholomo Drori, Information Officer of the Dead Sea Potash Works at S'dom, told *Nature*: "No one is putting pressure on the government for the important things, like a canal from the Mediterranean to the Dead Sea. The Southern part of the Dead Sea has vanished, probably for ever".

Professor Anthony Peranio of the Technion, renowned for his interest in renewable energy sources, said: "We should be building this canal. There should be great pressure brought to bear on the powers that be to get free-flowing water from the Mediterranean to the Dead Sea." Because the Dead Sea lies some 600 metres below the level of the Mediterranean, the canal, he said, could be harnessed for energy purposes, to generate electricity and cool inland power stations.

First French cosmonaut to be trained next year: Franco-Soviet space cooperation has taken a step forward with the announcement on 23 October that a French astronaut will be part of the crew of a Soviet spacecraft to be launched in the middle of 1982. This follows a meeting on space cooperation — the sixteenth such meeting in 30 years between France and the Soviet Union — in Corsica from 15 to 20 October. The meeting also agreed on details of a study of the atmosphere of Venus and reviewed the possibility of cooperation in biology, geophysics, meteorology and telecommunications.

"Truth in testing" legislation defeated in US: A bill requiring disclosure of the methods used by the companies which set and administer standardised educational tests has been defeated in the US Congress after an intensive lobbying effort by the testing industry. Tests such as the standard aptitude test (SAT) are set and administered by largely unregulated private firms. The tests determine the education of most American children from assignment to classes for the mentally retarded to college entrance, and are enormously influential. The so-called "truth in testing" legislation would have required (among other things) that the companies disclose examination questions. Higher education organisations and testing companies opposed the bill, claiming that disclosure would result in undue influence on school curricula. They also said it would be prohibitively expensive to prepare new questions for every exam and that the quality of questions would suffer. A proponent of the bill, Dan Cameron of the National Education Association, questioned the industry's motives. "The testing industry exerted all the influence it has to kill this legislation because they don't want to reveal what this legislation would make them reveal", he said.

● The industry suffered a defeat last week when a Federal judge ruled that standardised tests could not be used by California officials to determine the placement of black children in classes for the mentally retarded (*Nature* 25 October).

ISTC planners accused of illegal lobbying: Administration officials may have acted illegally by using funds appropriated by the US Congress to gather support from private individuals and institutions for the proposed Institute for Scientific and Technological Cooperation (ISTC), according to a report prepared by investigators for the Senate Appropriations Committee. The accusations have been passed on to the Department of Justice, and they could be the death-blow for the Institute, a centre piece of the US presentation at the United Nations Conference on Science and Technology for Development. The ISTC is now struggling desperately for congressional approval.

Three weeks ago, the US Senate agreed by 70 votes to 22 to cut all funds for the Institute out of the Foreign Aid Appropriations Bill. This was largely at the instigation of Senator Dennis Deconcini of Arizona, who earlier in the year had persuaded the Senate not to approve the ISTC's authorisation on the grounds that it would merely create another unnecessary government bureaucracy. The latter decision was later overturned in a conference with the House, and it had been hoped that the budget decision would also be reversed, since the House has already agreed to provide $23.75 million of new funds for the Institute. However the latest findings about the ISTC's lobbying activities have not increased its chances of success.

New crack in French reactor increases hazard: A further step in the crisis of the French nuclear programme has occurred with the revelation that there has been a leak of primary coolant into the secondary steam-generation circuit at the operational nuclear reactor Bugey-2 near Lyons. This was confirmed last week by the French trade union federation, the CFDT and is exactly what was feared might happen after the discovery of cracks, at 25 of France's 26 pressurised water reactors. The cracks were in the tubular collars that are supposed to seal off the radioactive primary coolant from the water circulating in the steam-generation areas (*Nature* 27 September) and mean that large sections of the reactor are now subject to radioactive contamination. The original discovery of cracks has already led to a strike and consequent postponement of the loading of two newly-built reactors (*Nature* 11 October). This time the state-run Electricité de France has refused to shut down the Bugey plant and has declared a moratorium on all public information unless previously cleared by government authorities. — *Jim Ritter.*

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Portrait of the out-of-work scientist

Government expenditure cuts are just statistics to some; to others they mean personal and professional tragedy. **Joe Schwartz** reports on those who are fighting back

Dr Anne Simmonds is an internationally known research biochemist specialising in purine metabolism. Forceful and competent, her problems as director of the Purine Laboratory of Guy's Hospital Medical School in London for the past eight years include convincing granting agencies that the study of a metabolism is as important as studies of specific diseases.

The field is a lively one. Studies of the loss of enzymatic links in the purine recycling process make specific interventions possible for at least five bizarre and life-threatening clinical abnormalities. One of these is the Lesch-Nyhan syndrome, a severe neurological disorder in children characterised by spasticity and self-mutilation which is due to a defect in one of the salvage enzymes of the purine cycle.

Simmonds' work, in conjunction with clinical colleagues, has led to her being "in the right place, at the right time, with the right expertise to be able to recognise a new disease for the first time". Her busy schedule includes the activities associated with an established international researcher: apointment to the Organising Committee for the Second and Third World Congress on Purine Metbolism in Man, invited participant from the UK to the Second World Symposium on Inborn Errors and Immune Disease in New York, and an appointment as Visiting Professor in the Department of Medicine at the University of Munich for a period early in 1980. Professor Fred Rosen of the Department of Pediatrics of Harvard Medical School describes the field as "bursting with excitement" and acknowledges Simmonds as having done "well known and very fine work with very widespread implications".

In September 1980, the Purine Laboratory will be closed and its Director, along with her staff of 4 graduates and one doctoral student will be unemployed.

Anne Simmonds is one of many senior researchers in the UK with international reputations and a long history of successful and productive research who are slowly being squeezed out of their research careers. Simmonds is vulnerable because she has moved through a succession of short-term contracts, the 'soft money' of the 60s, in order to be able to do her work. In the present period, research councils have found it convenient to regard short-term contract workers as dispensable and with 50% to 70% of the UK's basic research workers being supported by contracts researchers like Simmonds are caught in the net.

Simmonds' case is an important counter-example to official rhetoric about the effects of cuts on research programmes. Contract workers are protrayed as 'young and adaptable' or as old and 'burned out'. Official talk about the need to 'make room for new blood' or to 'maintain excellence' has muted criticism of recent policy decisions, by making a virtue out of the perceived necessity to cut public spending. Senior researchers who are unfortunate enough to find themselves in vulnerable positions have not only to face the loss of their jobs but also attacks on their competence for no longer being 'good enough' to warrant support.

Simmonds stresses that many scientists working in research full time on contracts have voluntarily given up other permanent jobs because of their commitment to research. In her case, she has turned down three permanent clinical jobs as well as a position at the Wellcome Laboratory in Beckenham. Her refusal of the Beckenham job ilustrates some of the problems facing researchers in the UK. Simmonds was hired in 1967 at the Wellcome to do purine research. But in 1970 Wellcome decided to transfer most of its purine programme to the US. Simmonds was offered a chance to go on to Wellcome's permanent staff but after accepting she found that the UK purine programme was being eliminated and that she would be required to work in immunology instead.

Through the intervention of Dr David Long, then Director of the Wellcome, and with funds provided, in true 19th century style, by an anonymous donor, Simmonds was able to set up a purine lab at Guy's in 1971. Her support from Wellcome stopped in 1972. She has since received a 3-year non-renewable contract from the MRC with the MRC making it "very plain" that they were not happy about a person of "advanced age (43 at the time) not having a permanent position." She was able to get a final three year bringing grant from the Wellcome in 1977 and with its termination in 1980 the work at Guy's will stop.

Simmonds has applied to "every conceivable" charity for funding from EEC funding agencies to the Cadbury, the Sainsbury and the Kellog foundations. She has not reapplied to the MRC. "If I was too old for them then, I am too old for them now. The MRC has always been less than helpful and we have either been ignored or forced to listen to insults about our capacity to research." As Simmonds puts it: "Because we have found it impossible to continue to do our work well under current pressures, we have dared to question our traditional role — and we have rocked the boat".

The MRC says that their records show that Simmonds had applied for and received a normal three year non-renewable grant in 1974 and has not applied since then. "The MRC has no concerns in this particular situation. She can apply for a grant as can everyone else and whether she does or not is entirely up to her. This is a matter for Guy's Hospital, not us", an MRC official told *Nature*. The loss of Simmonds lab means that there will be no work of international stature on purine metabolism in the UK.

Simmonds has had the energy and commitment to fight these pressures organisationally. She is one of the founding members of ARMS (Assosiation of Researchers in Medical Science) established in June 1978. "I was originally concerned because of the general problems facing staff: short term contracts, the loss of two years in every five or six writing and rewriting applications and the loss of younger people just when they have learned enough to be useful."

ARMS now has branches in both London and Manchester. It is attempting convince UK authorities that a career structure for non-medically qualified full time researchers is a long overdue necessity for the health of British medical research.

Simmonds describes her involvement in ARMS' campaign in personal terms: "Two of my senior colleagues with outstanding records, who, when ARMS was set up last year had 12 months left, are now out of work. This had happened despite the fact that they have actively contested their abandonment by the MRC after 13 years of short term support, both to that body and



Up in ARMS: committee members Sue Barlow, Mick Kadlubowski, Anne Simmonds, Arlene Unger

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to local authorities. Today I am in exactly the same position. I have one year left and my staff will have to follow me into the street since the school will not support them beyond the termination of my own grant." Simmonds intends to devote her political energies in the coming year to collecting data on the current treatment of senior scientists like herself. As far as her research goes she is more philosphical. "Although it is difficult to believe that there is no official recognition when one has done something as clinically useful as describing a new disease, we have to accept that we are in the wrong field with the wrong qualifications." □

Where MRC stands on jobs

THE Medical Research Council's current career structure for non-clinical scientific staff was agreed with the Association of University Teachers and came into force in 1974. It applies only to staff in the MRC's units and provides for three categories of appointment: three-year short term contracts, five year limited term appointments and tenured posts.

In general a new PhD would apply first for a three-year short term contract. At the age of about 27, after completing one three year term, he/she would then eligible to apply for a five year limited term appointment. At the end of that, at age about 31, he/she can apply for tenure.

The five year limited term appointment was created as a bridge between post-doctoral appointments supported by short term grants and tenured posts. The idea was that 75% of those with limited term appointments would receive tenure at the end of five years. Those who did not, would not normally be eligible for further three or five year appointments. This scheme was designed to prevent researchers continuing work on limited term grants into middle age by forcing them into a career decision in their early thirties.

The MRC currently employes 790 non-clinical scientific staff in its units. 21% of them are on short term contracts, 14% on limited term and 65% have unlimited tenure. The target figures, which the Council would hope to achieve after the system had been working for several years, are 18%, 15% and 67% respectively. However, with pressure on budgets due to public expenditure cuts, the MRC may never achieve these figures. It has recently been rethinking the career structure for non-clinical scientific staff and has put forward a proposal to the AUT for negotiation, which is likely to include a major modification of the tenure system.

The position in the universities is very different. The MRC provides tenured staff with grants for research projects. Untenured staff are paid out of these grants, the university being their employer. The MRC currently provides funds for 800-900 research studentships and 1300-1400 scientists on short term contracts. Half of the latter have PhDs and half have only first degrees.

Seven years ago, the MRC began to realise the problem of absorbing all these short term contract workers into permanent positions. It has, however, according to one official been surprised at how slow the crisis has been in coming. Many people have left the system by taking jobs overseas or moving right out of research. "The problem (of short term contract workers) will solve itself by the scientists making an appreciation themselves and then making the necessary adjustments". However, the Council does not know how far the process of adaptation has gone and criticises the AUT's attempts to quantify the problems. "The (AUT's) figure of 2500 unemployed scientists hasn't been substantiated and we don't really know if there is a problem, whether people have been able to adapt and whether able people have been discouraged from entering research".

The MRC's main response to the lack of tenured posts has been to create bridging fellowships to enable the "truly able" post-doctoral worker to wait longer than the normal five year limited term for the right tenured position to turn up. But the scheme has met with minimal response. The Council has also resisted pressure from within its own ranks and outside to recruit more graduate students.

Ideally, the MRC would like to have more control over research posts. Two MRC officials at least would like to judge the "hallowedness and timeliness" of research and to see a "population of mobile research workers who could move around at the Council's discretion". Another, Tony Vickers, told a meeting at the Royal Society of Medicine earlier this year that "it might not be a bad idea if we had to revert to immediate post-war conditions". This would mean a much smaller scale of scientific activity with no post-doctoral researchers. "Serendipity goes out of the window when postdoctoral workers come in".

The Association of Scientific, Technical and Managerial Staffs has been trying for several years to gain 50% membership on the MRC. Its attempts, however, have been resisted. It just wants "to turn Council into a private benefit society" according to one MRC official. "The Council is a self-perpetuating oligarchy" which has trust in its members who in turn have trust in it.

ASTMS, however, takes a different view. It considers fixed term contracts as a "primitive way of dealing with scientific and technical staff" which places "too much power in the hands of incompetent employers at a time of particular difficulties in scientific employment".

Judy Redfearn

SRC: university cuts are the greatest threat

"The science vote is not being harshly treated" by the Conservative government in the latest round of public expenditure cuts, according to Sir Geoffrey Allen, Chairman of the UK Science Research Council. There is a good case for maintaining a reasonable budget for research, he says, and this is having a strong influence on the government. The science budget is therefore unlikely to suffer the massive percentage cuts proposed for other areas of public expenditure such as education and many of the social services.

The SRC budget is to be cut by only 1½% in real terms in the current financial year and a further 2% in 1980-81. The lack of budget guidelines after 1981, however, is causing problems with medium term planning. For example, the Council's recently announced programmes in post graduate education to improve the performance of graduates in industry (*Nature* 2 August, page 347) are to receive priority over the next few years — but uncertainty in future budgets means that the rate of expansion of the programmes cannot be planned in advance.

Similarly, the SRC cannot plan space and astronomy beyond the end of the current programme in 1983. International collaboration may be one way of cutting costs, says Sir Geoffrey.

The greatest threat to the level of science funding in the UK, however, probably comes from the recently announced cuts in the universities budgets. "These are bound to affect science and engineering", says Sir Geoffrey especially in the short term when running costs will be squeezed. In the long term science and engineering will be cut proportionately less than other faculties and will therefore make a greater part of a smaller whole. "This could be good from our point of view. The government is saying that we've got to be more selective. This means either fewer universities or a smaller overall staff maintaining quality".

Sir Geoffrey hopes that the problem of providing employment for scientists (and coping with any redundancies the universities may have to make) will be helped by the SRC's research felowship scheme whereby senior scientists can vacate tenured posts by taking up SRC fellowship. "We have the money to do this on a small scale".

Sir Geoffrey's belief in the government's empathy for research was endorsed by the Prime Minister, Mrs Margaret Thatcher, herself last week when she told members of the Chemical Society of her committment to basic research at a ceremony to award her with the Society's Honorary Fellowship. "One must listen to the voices of scientists and pay attention to what they say", she said. □

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Thais put their faith in folk medicine

In first of two articles on scientific issues in Thailand, **David Bousfield** of the University of Sussex describes how religion and medicine often go hand in hand

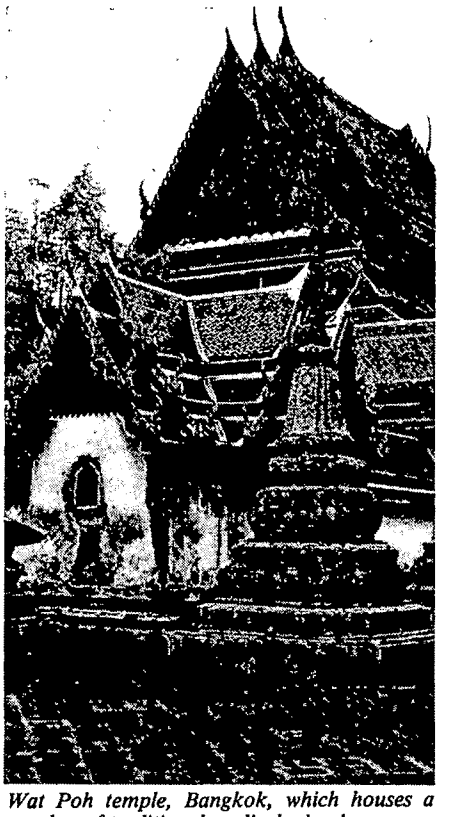
TRADITIONAL health care systems based on herbal preparations still exist in many developing countries today, and although often despised in the recent past they could provide a focus for the development of local pharmaceutical industries and research. Plants are already important sources of established drugs such as diosgenin (a precursor of many steroids), codeine and atropine. They may also contain compounds with novel actions. The massive screening of plant extracts for anti-cancer drugs carried out under the auspices of the National Cancer Institute, and the research currently being coordinated by the World Health Organisation on physiological bases of folklore contraceptives are good examples of the current interest in this field. In addition, by employing an indigenous health care structure, medical treatment may begin to penetrate rural areas where previously there was no help at all.

South-east Asia provides some useful examples of the possibilities and limitations of integrating this traditional knowledge with the medical needs of a developing society.

In Bangkok alone there are more than fifteen traditional medical schools, some of which can be found on the grounds of Buddhist temples. Wat Poh, the temple of the Reclining Buddha, for example, has long been an important centre for folk medicine in Thailand. In the provinces, such as Chiang Mai in the north, herbal pharmacies are common and are often found beside, or even inside, their modern counterparts.

Certain Buddhist monks, the 'maw phra' or 'doctor bhikku', play an important role in treating rural sick. In some cases these schemes can be very ambitious. The Abbot, Phra Chamroon Parn Chan, of the temple Wat Tham Krabok has recently been given the Ramon Magsaysay Award (a south-east Asian equivalent of a Nobel Prize, named after the late Filipino President) for his work with drug addicts. His approach relies heavily on herbal infusions "to clear poisonous drug residues from the body", thus eliminating "the physical desire for narcotics". With government subsidies the Phra hopes to be able to treat up to 4,000 addicts annually.

Doctor Bhikkus self-trained bare-foot 'tambol' doctors are very popular among the lower educated, lower income groups in both rural and urban districts. Several government training schemes are in existence which aim to improve this service in areas such as nutrition and family planning. Use of herbal medicines is taught in many university departments of pharmacy and pharmacology, and in some cases students may even attend additional courses at their local traditional school of medicine.



Wat Poh temple, Bangkok, which houses a number of traditional medical schools

Research is well under way on the extraction, identification and characterisation of the physiological, pharmacological and toxicological properties of the active principles present in a number of crude drugs. Particular areas of interest include the studies of alkaloids present in local *Rauwolfia* species, some of which could provide a commercial source of drugs such as reserpine. 'Kinchai', Chinese celery (*Anium graveolens*), is also being studied for its potent hypotensive actions and its dramatic ability to reduce temporarily sperm count in man. In Chiang Mai research is being carried out on extracts from a variety of plants including a *Curcuma* (Zingiberaceae), locally known as 'Wan Ngu'. This contains an inhibitor of cobra and viper neurotoxin, and the study of the properties of this extract and its active ingredients has stimulated an exciting amount of neurophysiological and neuropharmacological research. The universities of Mahidol and Chulalongkoru in Bangkok are carrying out even more intensive studies.

The development of useful drugs based on natural product 'diospyrol' serves as a good example. Roundworms, hookworms and other parasitic infections are a serious health problem throughout south-east Asia. In Bangkok's slums 2% of the children have *Ascaris* infections and 6% hookworm. In rural areas the prevalence of hookworm can exceed 80%, with anaemic complications in half the cases. Effective drugs for treatment do, of course, exist but are often unavailable in the more remote parts of the country. On the other hand, the tree 'Ma-gluea' (*Diospyros mollis*, Griff) which is indigenous to northern and central Thailand has long been recognised for the anthelminthic activity of its berries. The fresh fruit is crushed with a small amount of water, strained, and mixed with coconut water to mask its disagreeable taste.

Clinical trials have shown that the drug is effective against a wide variety of intestinal parasites, and the limited field testing which has been carried out has met with encouraging results. The plant, however, only bears fruit in the rainy season (June to October) and the remedy is only effective if the berries are fresh. A good deal of work, conducted mainly by the Ministry of Public Health in collaboration with Japan, has therefore gone into identifying the active principle, 'diospyrol', a methylated binaphthyl compound, and to discovering methods of preserving the extract. This work was completed in the late 1960s, but since then little has been done to develop herbal medicines to a commercial level.

Research into natural products and herbal medicines is being carried out on a similar scale in at least nine neighbouring countries. These are Thailand's four partners in ASEAN — Indonesia, Malaysia, Singapore and the Philippines — plus Japan, Korea, Hong Kong, Australia and New Zealand. Together they make up the Network for the Chemistry of Natural Products in South East Asia, a Bangkok-based organisation funded mainly by UNESCO and the Japanese government. It is, however, clear that serious problems occur whenever the commercial exploitation of a natural product medicine becomes a possibility.

Ironically this may be the result of the United Nations Industrial Development Organisation (UNIDO) plan for the establishment of drug industries in Third World countries, which involves a backward integration approach to drug manufacture. UNIDO feels that each country should begin with setting up packaging plants, then move on to formulation of imported bulk drugs, and finally manufactures the bulk drugs themselves. But in countries such as Thailand such an approach could stifle the motivation behind much of the current research into herbal medicines and result in many of these new compounds being developed and sold by the multinational pharmaceutical companies. □

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Population boom blunts Pakistan's self-sufficiency drive

Despite Pakistan's efforts to become self-sufficient in food grain, it is still falling short of the target every year. The resulting heavy expenditure on the import of wheat, the staple foodstuff, is compounded by considerable congestion at the port of Karachi and in trains carrying the millions of tons of wheat grain upcountry. The whole economy of Pakistan is suffering in consequence. Last year, for example, wheat created a crisis when the country had a bad harvest and imports rose to 2.3 million tons. The problem is not that production strategy is failing for a valid reason — such as targets being unrealistic or the potential being weak — but that the mechanism is inefficient and the will is missing for many imperative measures. These include the package of improved technology for different areas of Pakistan (a target now well within reach) and a streamlining of the distribution of seeds, fertilizers, pesticides and water to the farmer.

At the International Seminar on Wheat held in Islamabad last August, Dr Norman Borlaug, the Nobel laureate, said that at present only one-third of Pakistan's potential wheat production is being exploited. Borlaug, who has been intimately associated with the Green Revolution in Pakistan, blamed the existing official apparatus both for neglecting to apply the available improved technology to wheat production, and for the continued short-sighted reliance upon food aid and concessional purchases (under easy payment contracts from friendly countries, such as the US PL-480).

The pity is that Pakistan also happens to be one of the few countries that staged the Green Revolution early, in the mid-1960s. It had such tempo and potential that Borlaug at that time not only forecast self-sufficiency in food grain within a few years time, but also called Pakistan the 'Mexico of Asia', visualising that it would be exporting wheat and rice to other countries sooner than many imagined. Borlaug's dream was never realised; and sooner than elsewhere, the Green Revolution tapered off.

This is clearly borne out by the wheat production figures since 1965, when the dwarf, high-yield seeds from Mexico became available for commercial sowing.

It can be seen from the accompanying table that wheat production, after nearly doubling from 3.75 million tons in 1965 to 7.10 in 1969, has almost flattened off during the course of the last decade — last year even showing an alarming dip.

Self-sufficiency in wheat could have been attained, even with the very modest increase recorded in the last ten years, but for two inhibiting factors. One is the population explosion, running at a 3% per annum, a result of the slowly growing standard of living. The *per capita* daily wheat consumption has risen from 244 to 340 g during the last 10 years. Even with a record wheat harvest of almost 10 million tons this year, a short-fall of 0.6 million tons is still possible.

There is however hope that, unless the political situation deteriorates further, Pakistan may now be able to turn the corner. The new factor is a drastic reorganisation of the Pakistan Agricultural Research Council (PARC), which has turned it into a fully autonomous organisation with wide powers of planning, coordination, financing and formulating national strategy for agricultural research. The PARC now has a full-time chairman, with the status of secretary to the government. It is also intended that the should be a scientist of high repute and not a politician. The present chairman, Dr Amir Muhammad, has gained the necessary experience and expertise to organise research on a national level through his previous positions as Vice-chancellor of an agricultural university and Director of the Atomic Energy Agricultural Research Institute.

The PARC is now equipped to provide leadership to other research organisations in the field. This is particularly significant in Pakistan, where agricultural research has largely been fragmented at the provincial level and has lacked direction. This was all too manifest last year in the wheat production debacle. Farmers had sown considerable amounts of wheat varieties that were susceptible to disease, even though disease-resistant types could have been easily identified and the farmer suitably advised — if the research organisations and their provincial extension services had been alive to the situation.

This year's 20% increase in wheat output is partly due to the PARC reorganisation, and partly to the federal government itself. After recovering from the panic of having to import 2.3 million tons of wheat, and paying the heavy price, the government is now taking an active interest.



The PARC has started taking such vital steps as zoning the country for some of the major crops in terms of agro-ecological variations, sending its research teams in different zones to conduct investigations and research on the spot, and providing analytical service facilities not available at the provincial institutes. But its most significant achievement appears to be the establishment of properly constituted National Agricultural Research Centre near Islamabad. A staff of 25 scientists, eight of them postdoctoral, are already working at the centre, with another 40 in the process of recruitment. The emphasis is on high calibre to strengthen the team.

The centre has already mounted a large number of experiments on its own fields (which cover 1,500 acres) involving topics such as plant genetics, agronomy, and plant pathology. It is also investigating the 'package technology' of producing different grains for different areas. Each package will contain information on varieties, cultural practices, fertilizers, weed control, harvesting, and storage.

Genetic resources conservation is another vital responsibility of the centre. It has already collected thousands of exotic, as well as local, varieties of wheat, rice, triticale, maize and other grains. They will be preserved for up to 100 years in the main depository, in vacuum at 0°C. To mount an effective genetic variety programme, the breeding stock should have great genetic diversity; breeders all over the world have started to feel the pinch of starvation as new sources of genetic diversity are getting scarce, squeezed out by the rapidly spreading improved crop plant varieties.

Azim Kidwai

Table 1 Pakistan wheat production since 1965

Year	Wheat output (millions of tons)	Imports (millions of tons)	Population (millions; approximate)
1965	3.75	Not known	5.5
1968	6.50	0.015	
1969	7.10	0.123	
1971	6.78	0.439	6.5
1974	7.50	1.179	
1976	8.55	2.300	
1977	9.00	1.087	
1978	8.15	2.300	7.5
1979	9.99	Not yet known	

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news and views

Probing the insulin receptor

from P. H. Sönksen

THE German Wool Research Institute houses some of the foremost peptide chemists in the world who sharpen their teeth on insulin as a simple chemical model for the more complicated molecules that will be keeping us warm in the months to come. At the Institute two young students, Peter Thamm and Achim Schüttler have respectively synthesised a family of specifically photolabelled insulin analogues and made a variety of specific covalently-linked insulin dimers which together have led directly to major developments in our knowledge of the insulin receptor. The insulin receptor was perhaps the most popular subject at the International Insulin Conference* held at the Institute recently.

Remarkably concordant results on its composition were provided by three independent groups. M.H. Wisher and colleagues (St Thomas's Hospital Medical School, London) have specifically covalently labelled a membrane protein of approximately 300,000 molecular weight which can be resolved into a subunit of apparent molecular weight 130,000. This polypeptide runs atypically on polyacrylamide gels and may be a glycoprotein of true molecular weight nearer 90,000. Rather surprisingly this same membrane component was readily and specifically labelled with probes carrying the photoreactive group in the A_1, B_1 or B_{29} positions.

These observations were exactly in keeping with those of C. Yip (University of Toronto) working independently with similar but less specifically photolabelled probes. Yip reported two specifically labelled components (130,000 and 90,000 molecular weight) in membrane extracts from insulin-responsive cells but only the 130,000 molecular weight components in extracts of cells containing insulin binding sites but no insulin-responsiveness (such as brain) and went on to suggest that the 90,000 component may be an effector molecule. M.P. Czech (Brown University, Rhode Island) using a bifunctional cross-linking reagent was able to produce almost identical results by covalently linking radiolabelled insulin to the membrane receptor after the two had been allowed to react *in vitro*. He, like Wisher, had also isolated a 300,000 molecular weight component that could be dissociated into 125,000 molecular weight subunits after reduction of disulphide bonds. Early results from C. Diaconescu and D. Saunders (DWI) and A.R. Rees (University of Oxford) suggest that covalent linkage of insulin to its receptor in adipocytes may lead to prolonged activation of insulin-dependent processes.

Other remarkable observations on function of the receptor come from the synthetic dimers (Schüttler). They show considerable discrepancy between receptor-binding activity (100%) and biological activity (considerably impaired, 0–60% depending on assay). P. De Meyts (Institute of Cellular and Molecular Pathology, Brussels) and colleagues concluded, on the basis of their studies with these dimers, that each monomeric half of the dimers might bind to a component of the insulin receptor and in some way prevent activation (possibly by inhibiting subsequent conformational change). On the other hand K. Willey and colleagues (St Thomas's Hospital Medical School, London) felt that their own results were compatible with the simpler concept of dimer reaction through one component only.

Other examples of the important contribution made by the synthetic chemists include some very elegant experiments reported by A.S. Rosenthal (Merck Sharp and Dohme Research Laboratories, New Jersey).

In the presence of synthetic peptide fragments of insulin, ^{3}H-thymidine incorporation into lymphocyte DNA as a measure of T cell response, was used to delineate the specificity of the phenomenon. In one strain of guinea pigs

*The 2nd International Insulin Symposium was held at the Deutsches Wollforschungsinstitut, Aachen on 4-7 September, 1979, and organised by Professor H. Zahn and Dr Dietrich Brandenburg.

P.H. Sönksen is Professor of Endocrinology, St Thomas's Hospital Medical School, London.

More on anthropoid origins

from Peter Andrews

THE origin of the higher primates is a contentious issue at present. Traditional interpretations place the tarsier closest to the anthropoid primates but an alternative to this has been advocated for some years by P.D. Gingerich of the University of Michigan. Gingerich has demonstrated a nearly continuous series of fossil primates from the early Eocene to the Oligocene all belonging to the family Adapidae (for a review see *J. hum. Evol.* **6**, 483; 1977), and he has shown that these become adaptively similar with time to early anthropoids.

In this issue of *Nature* (page 65) a group of Burmese and American anthropologists have described a new specimen of fossil primate from the late Eocene of Burma. This is named *Pondaungia cotteri*, and it possesses a number of specialisations that suggest it had reached the anthropoid grade, specialisations such as the great depth of the lower jaw, and the cusp and wear patterns of the teeth. These specialisations are overlaid on a pre-anthropoid pattern that seems to be most similar to the generalised adapids of Europe and Asia that Gingerich advocates as the immediate precursors of the Anthropoidea, and if these conclusions are substantiated by further discoveries it might well be possible to locate the origin of the anthropoids in the late Eocene of Asia. Work is continuing in Burma, and it is to be hoped that Ba Maw and Russell Ciochon will find additional specimens.

By the middle of the succeeding period, the Oligocene, anthropoid primates are represented by a number of species in the Egyptian Fayum deposits, which are currently being excavated by Elwyn Simons of Duke University, and by the end of the Oligocene they are also known in tropical Africa. They diversified during the succeeding periods up to the present, giving rise to all the living species of monkeys and apes and man. The adapids on the other hand diminished in numbers after the end of the Eocene, with the latest known population occurring in 10 million year old deposits in India and Pakistan where they apparently existed as a relic population (described earlier this year by Gingerich in *Nature* **279**, 415; 1979).

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T cell response could be localised to a few amino acids around the tenth residue of the B chain. On the other hand the specificity of antibodies produced by these animals did not reflect the specific determinants unique to the insulin used for immunisation. This distinction between T cell response determinants and specificity at the serum antibody or B cell level was also shown in mice. □

Nuclear quadrupole resonance at Toulouse

from J.A.S. Smith

P. GRIVIT (Université de Paris), in whose laboratory the first nuclear quadrupole resonance experiments in France were conducted, opened the conference*. He reviewed in masterly fashion the many current applications at Orsay of nuclear quadrupole interactions to solid state physics, concluding with the description of a sensitive pulsed spectrometer designed by L. Guibé and his coworkers (Paris, Orsay), a feature of which was its highly automated operation. This theme was repeated in several other papers, notably from E.A.C. Lucken's group (University of Geneva) who described a pulsed FT spectrometer for the study of single crystals, from which the complete electric field gradient tensor may be derived under computer control. Other workers described frequency and phase-locked spectrometers for the determination of true line shapes (P.T. Narasimhan, Indian Institute of Technology, Kanpur) and a special pulsed coil system for detecting ^{14}N signals from samples external to the spectrometer (T. Hirschfeld & S.M. Klainer, University of California, Berkeley) with potential applications to geochemistry. A recent development, the derivation of nuclear quadrupole interactions in free radicals by ENDOR experiments, was described by A. Colligiani (CNR Quantum Chemistry Laboratory, Pisa), who reported the first measurement of the full ^{2}H quadrupole coupling tensor in the free radical $HOOC.\dot{C}D.COO^{-}$.

The applications of nuclear quadrupole resonance to transition metal chemistry were emphasised. T.B Brill (University of Delaware,) described the applications of ^{55}Mn quadrupole resonance to the study of the electronic structure of tricarbonylcyclohexadienylmanganese(I) and its substituted analogues, and other speakers discussed ^{63}Cu resonance in diamagnetic copper(II) complexes (T. Okuda & A. Weiss, Technical High School, Darmstadt), ^{35}Cl quadrupole resonance in mercury pentachlorocyclopentadienes (G. Wulfsberg and coworkers, St John's University, Collegeville, Minnesota), in some hexachloroplatinates (IV) hexahydrates (A. Sasane, Shinshu University, Japan), and Zeeman studies of ^{63}Cu resonance in single crystals of Cu(I) triphenylphosphine dimers (H. Negita *et al.* Hiroshima University, Japan). Although it has been known for some time that quadrupole resonance of ligand nuclei can be readily studied in paramagnetic complexes, for example ^{14}N quadrupole resonance in dichloro (dimethylnitrosoamine) copper (II) (D. Nakamura, Nagoya University, Japan), T.J. Bastow and H.J. Whitfield (CSIRO, Clayton, Australia) showed in their paper that strong ^{63}Cu signals can be detected in several paramagnetic Cu(II) salts such as $CuCl_2$ and $CuBr_2$; the signals are seen because of strong exchange narrowing, with widths of about 35 kHz, little different from that of ^{63}Cu resonance in diamagnetic Cu_2O. In a review of recent studies of inorganic coordination compounds, T.L. Brown (University of Illinois) discussed the wealth of information now forthcoming from solid complexes by a combination of conventional cw and pulsed measurements with double resonance techniques.

The remarkable increase in sensitivity afforded by double resonance experiments was emphasised by many speakers. For ^{2}H and ^{14}N, the technique is now well established; J.L. Ragle (University of Massachusetts, Amherst) demonstrated the usefulness of ^{2}H quadrupole resonance in a study of pyridine and its salts, and ^{14}N double resonance was applied by M. Garcia (Queen Elizabeth College, London) to many imidazole derivatives and by A. Loetz and J. Voitländer (University of Munich) to a series of amidinium salts. Three papers were devoted to another recent development of double resonance spectroscopy, that of the detection of ^{17}O quadrupole resonance signals in natural abundance; J.A.S. Smith (Queen Elizabeth College, London) and S.G.P. Brosnan and D.T. Edmonds (University of Oxford) discussed the novel features of such methods applied to compounds containing -O-H groups, the most important of which is the appearance of dipolar structure, and O. Lumpkin (University of California, San Diego) described its applications to compounds containing O-O bonds, such as $IrO_2Cl(CO)(PO_3)_2$ a reversible oxygen carrier. In a later paper, P.J. Bray (Brown University, Rhode Island) demonstrated very clearly the significance of ^{14}N quadrupole coupling constants as a measure of biological activity, for example in the bateriostatic activity of the sulphanilamides and the tumour-inhibiting properties of several nitrogen mustards.

Studies of phase transitions and molecular motion in solids were common themes of a number of papers. J.R. Brookeman (University of Florida) gave a timely review of the importance of measurements of the resonance frequency and relaxation times close to phase transitions and several other papers discussed the temperature dependence of the same quantities for example, in hydrazine derivatives (Y. Abe, University of Tsukuba, Japan), in some bromocyclotriphosphazatrienes (A.L. Porte, University of Glasgow) and in ammonium salts such as α-NH_4HgCl_3 and NH_4ReO_4 (H. Chihara, Osaka University, Japan) in which the anomalous temperature coefficients were interpreted by taking into account the finite jump-time of the NH_4^+ ion between its equilibrium configurations. There was increasing interest in the value of variable pressure studies in studying solid-state processes; R.J.C. Brown (Queen's University, Kingston, Canada) stressed the generality of the relationship between the temperature and pressure coefficients of the quadrupole resonance frequency and J. Stankowski (Poznań, Poland) studied ^{75}As resonance in $K D_2AsO_4$ up to 3kbar as a test of current theories of proton-ordering in this crystal. I.G. Shaposhnikov (Perm, USSR) discussed a new theoretical attempt to analyse the 'Bayer' problem, the temperature variation of the quadrupole spin-lattice relaxation time, T_{1Q}, due to random thermal oscillations, by the methods of quantum kinetics; his theory shows that significant departures of T_{1Q} from the predictions of the Bayer equations can be expected at temperatures above $T_{m/5}$, T_m being the temperature at which orientational order disappears. Experimental aspects of relaxation time measurements were referred to by A.H. Brunetti (IMAF, Cordoba, Argentina) and there was some discussion of the usefulness of T_2^*, the spin-phase memory time, by S. Hacobian (University of Sydney) and M.A. Whitehead and coworker (McGill University).

Nuclear quadrupole resonance spectroscopists have long been worried about high-order nuclear electrical multipole contributions to their measured frequencies; S.L. Segel (Queen's University, Kingston) showed by careful frequency measurements on a number of nuclei such as $^{185,187}Re$ in NH_4ReO_4, ^{209}Bi in $BiCl_3$ and ^{93}Nb in $NbCl_5$ that there were no measurable hexadecapole contributions. The question as to whether this is generally true still remains. □

*The fifth international symposium on Nuclear Quadrupole Resonance Spectroscopy was held in Toulouse, France on 10-14 September, 1979. The co-chairmen were L. Guibé (Université de Paris) and G. Jugie (CNR, Toulouse).

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Urban ecology

from Peter D. Moore

THERE are areas of ecology where the development of theoretical models has outpaced the accumulation of the field data necessary to test them. Often the observations which exist are not in the appropriate form, and models stand unchallenged until the required information is gathered. One conceptual model which has received much attention from various quarters in recent years is the idea of island biogeography and its applicability to fragmented habitats of all kinds. The original statements concerning the immigration, extinction and stabilisation of island biotas were tested experimentally by Simberloff and Wilson (*Ecology* **50**, 267; 1969), but since then the model has been used extensively in ecology, for example in the determination of the size and spacing of nature reserves (see May *Nature* **254**, 177; 1975). Although, theoretically the model can be used in any fragmented habitat where intervening areas are unsuitable for settlement, experimental or even detailed observation concerning its wider applicability are generally few. Some such applications are mountain tops (Brown *Am. Nat.* **105**, 467; 1971), fresh water habitats (Sepkoski & Rex *Syst Zool.* **23**, 165; 1974), oak trees and leaf-mining insects (Opler *Am. Scient.* **62**, 67; 1974) and cushion plants (Tepedino & Stanton *Oecologia* **25**, 243; 1976).

One type of habitat in which island biogeographic theory could be put to the test is the mosaic of wasteland produced by demolition in city centres. Here are sites which are available for colonisation by weeds from surrounding sources, invading across unfavourable intervening terrain. Often the age of such sites can be precisely established and it could be argued that a level of equilibrium between immigration and extinction should be established relatively rapidly. If this were so, then theoretical considerations would lead one to postulate that the number of species at a site should be a function of the site area and should be inversely related to the distance from the origins of immigrant flora.

Crowe (*J. Biogeog.* **6**, 169; 1979) has examined the potential of such sites in Chicago. He surveyed 26 vacant urban lots in the city, establishing a plant species list for each site in addition to the determination of lot area, age, distance to the nearest older lot and lot density in the area, among other parameters. A disadvantage of a single survey rather than a long term study of each site is that one cannot be sure that species equilibration has occurred; nothing is known of the actual pattern of immigration and extinction. Crowe demonstrates that the vast majority of species found in the younger lots have survived in older sites also, so the system has not reached a successional stage where competitive processes are leading to extinction. This is further confirmed by the fact that the species number in a lot is closely related to site age in the younger lots, but for older ones (greater than four years) there is little increase with time. This indicates, but does not fully prove, species equilibration.

The number of species in these older equilibrated lots is positively correlated with lot area and to the density of other lots in the vicinity (number of lots within 1 km). It is negatively related to the distance to the nearest lot. These two latter features are indices of isolation from potential sources of immigrant plants. Thus the conclusions reached are in keeping with the predictions of island biogeographic theory.

One further variable which requires consideration is the diversity of microhabitats available within any lot. Crowe has not attempted any such analysis, but this aspect of the island biogeographic model has been looked into by Simberloff (*Ecology* **57**, 629; 1976), who concluded that although habitat diversity is an additional complicating factor, if one can eliminate this variable experimentally there remains a residual relationship between number and island area.

Observational data, however, may not permit such a fine resolution of the influence of island area and habitat diversity, for the two are frequently themselves positively related. Thus, Juvik and Armstrong (*J. Biogeog.* **6**, 205; 1979) when examining the biogeography of the endemic honeycreepers (Drepanididae) of the Hawaiian archipelago, found a positive correlation between island area and species richness. But since larger islands are also more diverse in their habitats, generally having the higher mountains and more

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Nutrient retention in tropical rainforests

from Robert M. May

IN the hour you might devote to reading this issue of *Nature*, more than 3,000 acres of tropical rainforest will be destroyed. According to the Food and Agriculture Organization of the United Nations, there were about 16 million km^2 of rainforest around the turn of the century, which by 1975 was reduced to 9.4 million km^2, representing a 40% reduction; a conservative estimate of the current loss rate is 27 million acres per annum, or 50 acres per minute. This mounting tragedy of the world's rainforests is an oft-told tale (two recent and powerful articles are by Lovejoy and Colinvaux in *Nature Conservancy News* **29**(4), 1979; see also *Nature* **257**, 737; 1975), and I intend here to dwell only on one narrow aspect of it.

Many lowland rainforests grow on very poor soils. These acid soils have low capacity for ion absorption, making them particularly susceptible to the leaching of all plant nutrients. Rainforest trees have responded to this by storing most of the nutrients of the forest in the trees themselves, and by evolving symbiotic associations with mycorrhizal and other fungi that recover and recycle those nutrients that make their way into the soil when a tree dies or when its parts are eaten and excreted by animals. Colinvaux extols these fungi, "Whose pervading presence contributes to that characteristic greenhouse smell of the forest. The smell of the fungi is the smell of life — the part of the system that circulates nutrients. When a rainforest is cleared, the smell is gone, and so are the nutrients that fungi and rootlets once conserved to make productive life possible."

Despite their importance, these ideas about direct cycling of nutrients in rainforests have only recently received direct experimental verification. In a most elegant study, Stark and Jordan (*Ecology* **59**, 434; 1978) added radioactive ^{45}Ca and ^{32}P to the surface of experimental plots in the Amazonian rainforest, near San Carlos on the Rio Negro in Venezuela. These radioactively labelled nutrients were added both in leaves and twigs placed on the forest floor, and as a direct chemical spray (simulating 'decomposed' litter) on the plots. The fraction of this radioactive material that leached through the root mat and humus layer was monitored for 6 months. In 9 of the 10 study sites, less than 0.1% — one part in a thousand — of the labelled nutrient leached past the root mat, and all leaching ceased after 1 to 2 months; in one of the 10 plots, slightly less than 1% of the radioactive material leached through. In addition, Stark and Jordan analysed root mat samples, showing directly that the radioisotopes were indeed taken up and translocated by living roots.

Noting that the history of the Amazon Basin and other tropical rainforests is filled with failure after failure to establish large-scale agriculture, tree plantations or pasture, Stark and Jordan are concerned to emphasise the message substantiated by their experiments: "The exploiters reason that an area that can support a luxurious forest should be able to support permanent agriculture. This study has demonstrated that this is not the case, because of the evolved and unique root mat which enables the undisturbed forest to survive." □

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'ecozone patches', it proved impossible to extricate the pure area influence from that of habitat heterogeneity. Evidently this aspect of the island biogeographic model needs more attention in order to understand the area/richness relationship whether at the urban lot or the Hawaiian island level of organisation. □

Mapping the nuclear potential

from P.E. Hodgson

THE special characteristics of nuclear heavy-ion interactions enable the nuclear surface to be probed in detail, and this has recently been used by Nees and Guidry of Oak Ridge and Donangelo and Rasmussen of Berkeley (*Phys. Lett.* **85B**, 201; 1979) to map the deformed potential of the nucleus ^{160}Gd.

To do this, they analyse the inelastic scattering of ^{40}Ar by ^{160}Gd at energies comparable with that of the Coulomb barrier. This interaction excites several states in the ground-state band, and there is strong interference between the Coulomb and the nuclear forces. At these energies, the wavelengths of the ions are small, so that semi-classical theory can be used to understand the interaction. This shows that since both Coulomb and nuclear forces contribute to the excitation, both the charge and the matter distributions influence the interaction, and are thus both probed by it. At energies below the Coulomb barrier few non-elastic processes take place, and so the effects of the real and imaginary parts of the potential can easily be separated.

From the uncertainty principle we know that localisation in angle requires a large uncertainty in the momentum. If we consider the experimental situation as shown in Fig. 1, when scattering is restricted to backward angles, we find that the transfer of multiple rotational quanta implies a localisation in the orientation angle of the deformed rotor during the interaction, and hence a localisation in the part of the nuclear surface probed by the projectile. Classical phase space arguments show that each classical angular momentum I_f is associated with a range of initial orientations obtained by projecting two units of I_f on the angular axis, as shown in the figure. This spread in spin corresponds to a definite range of initial orientation angles, so that a particular spin state probes specific regions of the deformed nuclear surface.

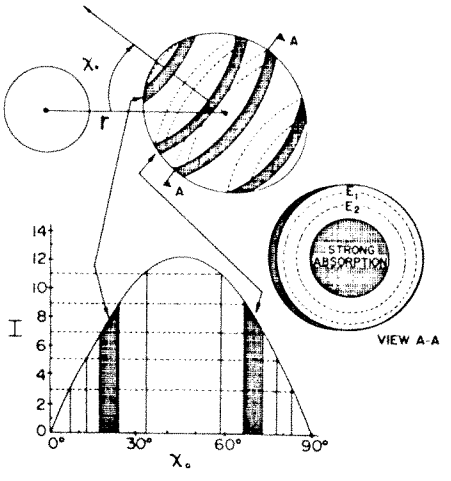


Fig. 1 Schematic illustration of the radial and angular localisation in heavy ion reactions. The more classical the system the more the surface interaction is localised in angle and in radial distance.

Similar arguments show that a large radial angular momentum implies localisation in the radial coordinate. Thus the initial width of the radial wave packet for ^{40}Ar on a heavy nucleus at the energies of these measurements is about 0.1 fm. The projectile spends most time in the region of the classical turning point, and so the interaction probes this region of the nuclear surface. As the incident energy increases the turning point moves inwards and with it the region being probed, until the particle is absorbed by the strong interaction in the nuclear interior.

These qualitative arguments are of course confirmed by numerical calculations that are fitted to the cross-sections for the inelastic scattering with the excitation of the low-lying states of the ground-state rotational band. A complex deformed Saxon-Woods potential was used, with fixed potential depths, and the radius and diffuseness parameters refer only to the localised region probed by the particular interaction.

From the results of these calculations the real part of the potential is constructed piece by piece, assuming axial and reflection symmetry. It is found that for each quadrant the classically allowed states (4^+ and 6^+) sample two regions while the classically forbidden ones (8^+ and 10^+) sample one broad region. The first three diagrams in the figure show the partial contributions of each state, and the final one the sum. It is notable that the contours overlap and join very smoothly, even though each comes from an independent analysis of a different part of the potential.

These potential contours can easily be parametrised in terms of an expansion in spherical harmonics to give a precise description in the usual form. They are closely related to the contours of the nuclear density distribution itself through a simple folding calculation with the nuclear interaction. Conversely, they may be used to check the accuracy of any particular form of the nuclear surface. This method of analysis of heavy ion interaction thus gives a very direct and clear description of what it means for a nucleus to be deformed, and should have many useful applications to studies of nuclear structure. □

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Bird colours

from John R. Krebs

MOST biologists, if asked to hazard a guess about the possible significance of the brilliant plumage adornments of many bird species, would probably say that bright colours evolved in the context of ritualised display between rival males or as attractants to lure females. They would point to the fact that male birds are usually brighter than females, and exotic male plumage is especially highly developed in polygamous birds. Males generally compete for females, and this competition is more intense in species where one male can acquire a larger number of mates, so sexually selected colours are expected to be found in males, and especially in polygamous males. This was the line of argument put forward by Darwin, and although a number of alternatives have been proposed from time to time, the first serious attempt to examine Darwin's idea by means of a large scale comparative survey has recently been published by Baker and Parker (*Phil. Trans. R. Soc.*, **B287**, 63-120; 1979). As a result of their analysis, Baker and Parker reject the sexual selection hypothesis and replace it with what at first sight may seem a surprising idea: that bright colours have evolved in response to selection pressures exerted by predators.

More than 30 years ago, Cott published an extensive study showing that bright coloured birds are often distasteful (*Proc. Zool. Soc., Lond.* **116**, 371-524; 1946). His work started with an observation made while preparing museum skins in the Middle East. Cott noticed that hornets readily ate the flesh of cryptic birds such as the palm dove (*Streptopelia senegalensis*) and ignored the carcass of a conspicuous pied kingfisher (*Ceryle rudis*). This lead Cott to a study of the hornet's preferences for the flesh of 38 Middle Eastern birds ranging from the very cryptic wryneck (*Jynx torquata*) to the conspicuous white crowned black wheatear (*Oenanthex leucopyga*). These, and later tests with cats, and a survey of human gastronomic preferences, confirmed Cott's thesis that bright colours are often associated with unpalatability. The phenomenon of warning (aposematic) colouration is well known in insects, and Cott extended the idea to birds. Baker and Parker point out that distastefulness is not the only basis for aposematic colouration: if a prey item is hard to catch, bright colours may evolve as a signal of unprofitability. They suggest that this might be especially applicable to birds, which once they have seen a predator can manoeuvre fast enough to be almost certain of escape. Thus the bright colours of many birds might signal not only "I am distasteful" but also (or alternatively) "I am hard to catch" or "I have seen you". As with the conventional theory of aposematic colouration, Baker and Parker's thesis assumes that bright, as opposed to

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Structure and function of eukaryotic genes

by Jonathan Wolfe

FIVE years ago few biologists would have predicted that the structural organisation of eukaryotic genes would be known in such great detail. However, although we now know the primary structure of several genes, the mechanisms by which their transcription is controlled remain unclear. Speakers at the fifteenth Harden conference* set out to bridge this gap between our knowledge of structure and its relation to function.

One system which we are on the way to understanding is the transcription and processing of yeast tRNA genes. The primary transcript of these genes is trimmed at both 5′ and 3′ ends and a short intervening sequence is removed to produce the mature tRNA. J. Abelson (University of California, San Diego) reported work in progress to purify the enzymes responsible for splicing out the intervening sequence, using as a substrate precursors purified from a temperature-sensitive mutant strain deficient in processing. A specific endonuclease and ligase have been separated, which are unusual in that they respectively create and rejoin a 3′ terminal phosphate and a 5′ hydroxy group. E. de Robertis (MRC Molecular Biology Laboratory, Cambridge) reminded us that tRNA precursors microinjected into *Xenopus* oocytes can be completely processed (*Nature* **278**, 137: 1979). That toad enzymes can correctly splice out the yeast tRNA intervening sequence is surprising since it has diverged markedly in sequence from that of *Xenopus*. B. Hall (University of Washington, Seattle) has taken advantage of the ability of *Xenopus* enzymes to process yeast tRNA correctly to probe the relationship between structure and function, by studying the transcription and processing of cloned mutant tRNAs in a *Xenopus* cell-free system. Twenty mutants selected by loss of nonsense suppressor activity, have been cloned and sequenced. Interestingly, all were found to lie within the 5′ and 3′ limits of the mature tRNA. One mutant, located in the intervening sequence, was not correctly transcribed and gave a primary transcript only 48 nucleotides long.

This type of 'surrogate' genetics can clearly provide information about the influence of primary structure on transcription and processing; however results obtained in heterologous systems should be interpreted cautiously. J. Beggs (Plant Breeding Institute, Cambridge) showed us that not all yeast and vertebrate systems are mutually compatible. She transformed yeast with a plasmid carrying the rabbit globin genes and flanking sequences. Globin transcripts were made: however the yeast did not splice out the first intervening sequence and transcription terminated within the second. This point was also made by C. Weissmann, in the Harden Lecture, who stressed the value of studying transcription and translation in a homologous system.

The yeast tRNA genes were not the sole topic of the meeting. C. Henschel (University of Zurich) reported results from M. Birnstiel's group who have now almost completely sequenced the sea urchin histone genes. They have found a Hogness box, the putative eukaryotic promoter, a short distance upstream of the H2A gene and have constructed a number of plasmids carrying small deletion mutants of this box or of the surrounding sequences. Sea urchin DNA supercoils, removed from the plasmid vector, were then tested (in *Xenopus* oocytes) for their ability to be transcribed and translated. Deletion of sequences between the Hogness box and the AUG codon of the H2A mRNA was found to have little effect on the amount of transcription. However, removal of the Hogness box greatly reduced transcription and led to variation in the point of initiation. What, at first sight, were contradictory results were related by R. Kamen (Imperial Cancer Research Fund, London) reporting work with a deletion mutant isolated by M. Bendig and W. Folk of the University of Michigan. He told us that deletion of an apparent Hogness box upsteam of the polyoma virus early region genes actually led to an increase in the rate of transcription initiated at a new site upstream of the deletion. However, the deletion extended into the 5′ end of the message and may have decreased the rate at which it was translated. One possible explanation of this result was therefore that the early region is under feedback control exercised by one of the protein products and the reduction in this inhibition may more than cancel out any possible decrease in transcription as a result of deletion of the promoter.

Surrogate genetics is clearly a powerful tool to investigate structure-function relationships in straightforward systems but it is of limited value when probing more complex situations. However, we were provided with one example of the rapid progress which molecular biologists can make when working with a complex system already well-characterised genetically. K. Nasmyth (University of Washington, Seattle) reported work in which he has confirmed the geneticists' 'cassette'model of yeast mating type switching. This model explains the 'switch' in mating type from α to a or *vice versa* which can occur in budding yeasts, by proposing that silent copies of the two mating type genes exist elsewhere in the genome (at loci HML$\alpha$and HMRa) and that they can reprogramme the locus at which the active mating type is expressed (MAT). Nasmyth has now shown common sequences at the MAT locus and at the loci HMLα and HMRa. Clones were selected for their ability to complement MATα^- yeast, which are unable to respond to mating type a hormones and it was then straightforward to clone all three loci. Heteroduplex mapping showed that although the clones had sequences in common they contained a or α-specific sequences and also sequences specific to each locus. □

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*Held at Wye College, Ashford, Kent UK on 16-21 September, 1979, and organised by Professor Peter Walker on behalf of the Biochemical Society.

dull, colours are needed to signal a warning, (although there is no evidence that predators learn faster to avoid bright colours). There is also a possible problem in explaining how the genes for warning colour spread in the first place, since each predator has to attack, and perhaps kill, a few individuals to learn its lesson.

Baker and Parker also suggest that some bird colours are used as 'flash colouration'. These are colours of otherwise cryptic animals which are exposed suddenly when a predator attacks. The predator is temporarily startled or confused, allowing its potential victim to escape. The bright red eye and black and white head of the night heron (*Nycticorax nycticorax*) might be such an example.

The evidence adduced by Baker and Parker for their hypothesis consists of a comparative survey of 516 species of western Palaearctic birds. For each species they coded conspicuousness on a scale of 0–5 for different regions of the body — crown, head, neck, back, belly and so on. They then carried out a stepwise multiple regression to look for correlations between conspicuousness of each body region and various ecological and life history variables such as diet, gregariousness, mating system, migration distance, and nesting behaviour. The analysis was done separately for adults, juveniles, males, females, summer and winter plumage. The authors admit that there are many inadequacies in their analysis, not least of which is deciding how conspicuous a bird is in its natural habitat.

I can mention only a few of the many

results produced by the analysis. As expected, males emerged as more conspicuous than females, and the dimorphism was more marked in polygamous than monogamous birds. These results seem to fit nicely with the classical sexual selection hypothesis, but according to Baker and Parker they do not. For example, within the polygamous species, only those in which the males display on open ground with a good view of approaching predators are bright coloured. This might be seen as supporting the idea that the bright plumage signals to the predator "I have seen you". Crypsis of females is generally related to their greater role in parental duties, and where males perform parental care they also tend to be cryptic, at least in the parts of the body most visible to predators. Because an incubating bird is more likely to be caught by surprise, it relies on crypsis rather than aposematic colours to escape predation.

A similar argument is used by Baker and Parker to account for the difference between diurnal and nocturnal birds. Diurnal birds are more conspicuous because they are usually alert to the approach of predators and are therefore unprofitable prey. Nocturnal birds rely on immobility and crypsis to avoid being eaten while they are asleep during the day, but they sometimes have flash colours which are revealed when the resting bird is disturbed. In this respect there is a parallel between birds and Lepidoptera, where diurnal forms are bright coloured and nocturnal species cryptic.

As with any comparative study of this sort, a major problem in the interpretation of Baker and Parker's results is to distinguish between cause and effect. Consider, for example, two polygamous species, the ruff (*Philomachus pugnax*) and wren (*Troglodytes troglodytes*). The former is strongly dimorphic and lives in open habitats where the conspicuous males display on communal ground. The wren is monomorphic and the cryptic males defend large territories in dense vegetation. According to Baker and Parker's thesis, male ruffs, as a result of living communally in open areas, are vigilant and therefore unprofitable to predators. Their bright plumage advertises their unprofitability. Male wrens in dense vegetation are easily surprised by a predator, and hence rely on furtive habits and crypsis to survive. But the interpretation can easily be turned the other way round. Suppose bright colours are always favoured by sexual selection, but that predation acts as a constraint to prevent sexually selected colours developing in some species. In the vigilant ruff the balance of these opposing selection pressures is in favour of sexual selection, while in the more vulnerable wren, predator selection has the overriding influence.

Baker and Parker restrict their analyses to overall differences between species in conspicuousness of particular body regions. Equally intriguing are remarkable convergences in the fine detail of plumage between unrelated species occupying similar ecological niches in different parts of the world. The African yellow throated longclaw (*Macronyx croceus*) and the North American meadowlark (*Sturnella magna*) constitute perhaps the most remarkable pair (Fig. 1), but there are many others. It is tempting to conclude that ecological selection pressures must influence the fine detail of plumage as well as overall conspicuousness, and a comparative survey like that of Baker and Parker could be a way of testing this conclusion. □

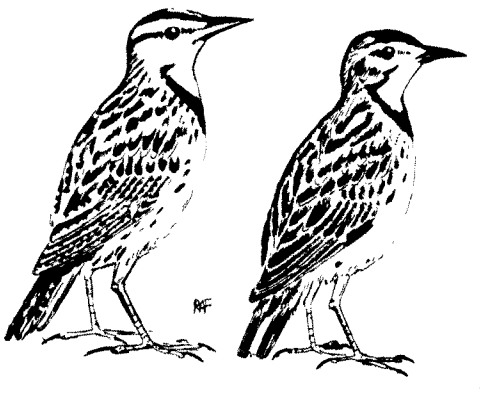


Fig. 1 Remarkable plumage convergence. The eastern meadowlark (left) is an American Icterid, while the yellow throated longclaw (right) is an African Motacillid, entirely unrelated.

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100 years ago

Several German newspapers strongly deprecate the undignified tone which seems to have reigned amongst the participators in the recent celebration at Pompeii. It appears that the majority of the visitors treated the whole matter as an excellent joke, and behaved very much as they would have done at a fair or at some other Neapolitan *fête*. Many of the archaeologists who had come long distances to be present at the celebration, left the dead city in disgust at the behaviour of the multitude, long before the proceedings were half over. One of the articles referred to expresses the hope that in the year 1979 the Neapolitans will have sufficiently improved in manners as well as gained in seriousness, to render a repetition of the digraceful scene impossible.

The London correspondent of the *Scotsman*, who is usually particularly well informed, states the Treasury has definitely decided not to ask Parliament for the cost of fitting up the new Natural History Museum at South Kensington and for the removal of the Natural History Department of the British Museum thereto till 1881. We are glad to be assured, however, that this statement should only be received as a rumour, and is not yet sufficiently authenticated. Let us trust that it will not be authenticated at all.

For the first time perhaps in the history of electric lighting two rival magneto-electric machines are illuminating the same hall. The Lontin and Siemens generators and lights are exhibited at about **120** yards from each other in the large hall of the Palais de l'Industri, at Paris. Each electrical machine is worked by steam, and consumes a certain amount of horsepower. The competition is too recent to offer yet any definite opinion on the respective merits of the apparatus confronting each other.

Telegrams from Murcia state that the city and celebrated *Muerta* were inundated by an immense waterspout which was formed in the sea at a distance from the coast on the night of October 14-15. Salt water was discovered at a distance of 45 kilometres from the sea. Another great atmospherical commotion was experienced three days afterwards. A night snowstorm enveloped the whole of Austria and Switzerland, and in Vienna the thickness of the snowfall was several inches. It is the first time since 1852 that snow has fallen so early in Vienna, but not the earliest time on record, as in 1760 it fell on October 12.

Mr. C. P. Edison, nephew of the great American inventor, has just died at Paris at the early age of twenty-four. He was his uncle's principal assistant in the production of the loud-speaking telephone, and was sent over to London by him to exhibit that instrument before the Prince of Wales, the Royal Society, &c. He had of late been engaged in applying his uncle's system of quadruplex telegraphy between Paris and Brussels.

The sanitary congress

This Congress continued its meetings at Croydon during last week, when several interesting addresses were given. Mr Douglas Galton, in his address last Thursday, spoke of that large class of conditions which are the direct result of the circumstances to which man is exposed in consequence of living in communities. All living beings are in a continual condition of change, which results in their throwing off from the body matters which poison earth, air, and water, unless space, time, and opportunity are afforded for the counteraction of these deleterious effects. He showed how thus resulted both epidemic and zymotic diseases, the presence or absence of which in any locality, and the degree of their virulence depend on the sanitary surroundings. Cholera and dysentery are principally connected with the condition of the water supply; while an epidemic prevails the questions whether a given population shall suffer or escape may almost be predicted from a chemical analysis of the drinking water. It is to the physiologist and the chemist that we must look for the causes from which these baneful effects arise, and what are the conditions which should be altered to prevent or remove them. The engineer steps in after these causes have been pointed out, and it is for him to design the methods of prevention or removal.

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articles

Relevance of Afar seismicity and volcanism to the mechanics of accreting plate boundaries

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The seismicity, the horizontal and vertical ground displacements, and the geometry of active faults and fissures during the seismic and volcanic events of November 1978 in Southern Afar are consistent with an accentuation of the pre-existing topography of the Ghoubbet–Asal rift. The zone of sizeable permanent deformation is restricted to several hundred square kilometres. Elastic rebound models seem to explain the observations. The events occurred in the vicinity of the suggested location of the tip of a lithospheric crack which may have been responsible for the progressive opening of the western Gulf of Aden.

AN important seismic swarm started on 6 November 1978 in the subaerial part of the rift which links Ghoubbet al Kharab to Lake Asal in the Republic of Djibouti (Fig. 1). North-west–south-east trending faults and fissures were reactivated in the inner floor zone of the rift, over a width of 3–4 km and visible length of 15 km, and a new volcano, Ardoukoba, came into being between 7 and 14 November. The whole sequence of events took place in an intensely studied section of the world ridge system, one of the few such sections that stand above water. This paper describes the seismic and tectonic aspects of the events and summarises data, much of which have been previously published only in French. We attempt to relate the events to the structural environment of Southern Afar and to test their relevance to geodynamical models of the Red Sea–Gulf of Aden plate boundary. More detailed accounts of the various observations and measurements will be published elsewhere.

Seismicity

The seismic swarm started on 6 November 1978, and lasted for 2 months, compared with only 1 week for the volcanic eruption[1]. It was recorded by the seismological network of eight stations, most of which have been operating since 1972. Only three stations were operating at the beginning of the swarm, but all stations were back in use by 10 November. After a first increase in seismicity on 6 November when two earthquakes with $m_b > 4$ were recorded, the climax occurred on 7 and 8 November. On 7 November, 15 earthquakes had $m_b > 4$ and the major shock reached a m_b of 5.3. This event was located in the axial part of the Ghoubbet, not far from the coast (Fig. 1). On 8 November eight shocks with $m_b > 4$ and another one reaching $m_b = 5$ were recorded. From 7 to 10 November, the daily number of recorded events with $m_b > 0$ exceeded 2,000.

Most of the earthquakes that have been located so far occurred along the axial fault zone in the Ghoubbet; preliminary fault plane solutions suggest normal faulting, in agreement with observed submarine scarps. A composite fault plane solution from a series of earlier earthquakes gave a similar result (refs 2, 3 and Fig. 2). There was little seismic activity in the subaerial section of the rift where the volcanic eruption occurred. The main locus of seismicity migrated from west to east with time through the Ghoubbet straights to the Arta transform fault zone, following closely the active plate boundary whose detailed geometry is now reasonably well understood (refs 4–8 and Figs 1, 2). This eastward migration pattern is thus very similar to that observed in 1973 during a previous swarm in the Gulf of Tadjourah[9].

Geodetic measurements and levelling

In 1972–73, a network of 22 geodetic stations was emplaced around the active Asal–Ghoubbet area[10]. Immediately following the November 1978 events nine stations were remeasured (Fig. 1). These measurements demonstrate that between 1973 and 1978 a large amount (at least 1.6 m) of extension occurred perpendicular to the rift axis. To establish the characteristic dimensions ('wavelength') of the deformed zone, a second set of measurements of further geodetic points was made: the additional data[11,13], together with the levelling data to be discussed shortly, show that the zone of the deformation does not greatly exceed the width of the inner rift (of the order of 10 km). Tarantola, Ruegg and Lépine[14] have computed the principal directions of strain which are compatible with the observations. They find two narrow bands of contraction (strains of the order of 10^{-4}) that border the axial part of the rift (4 km) where north-east–south-west expansion dominates (with strains of the

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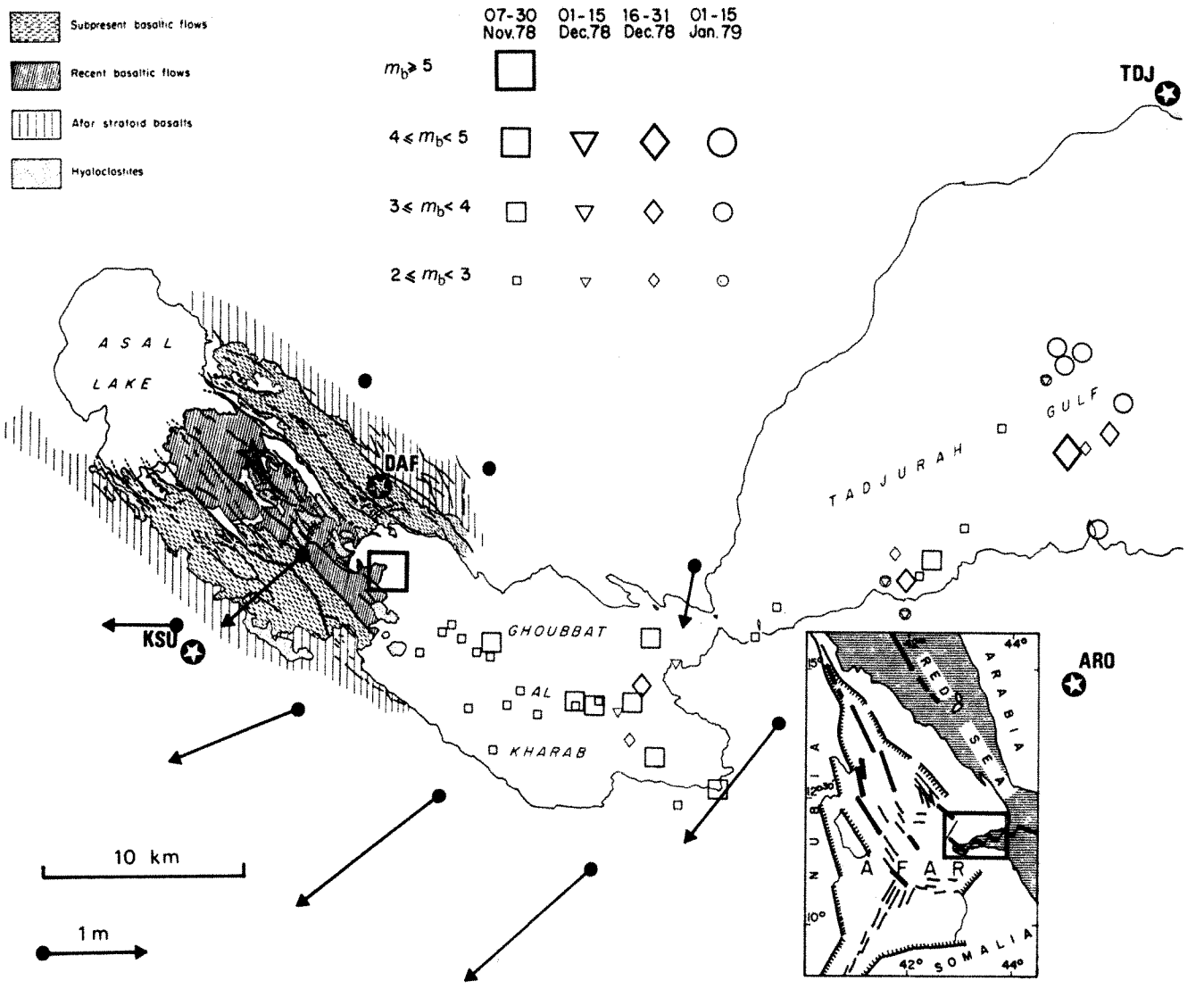


Fig. 1 Geological sketch of the Ghoubbet–Asal rift, evolution of seismicity from November 1978 to January 1979 (magnitude m_b and time of occurrence are given), and horizontal displacements (1973–78) measured at nine stations (the two northernmost stations are taken as reference, see refs 11, 12). Scales for displacements and for the geographical map are also shown. ☆, The November 1978 locus of volcanic activity; ⊛, some seismological stations. The general tectonic location is shown in the inset.

order of 2×10^{-4}). Computed strains seem to decrease with distance away from the Ghoubbet-Asal and become $<10^{-5}$ ~ 10 km from the axis.

In addition to the geodetic measurements, relevelling of a profile across the Ardoukoba rift, which had been measured first in 1972[10] reveals that, in the central part of the inner floor, a zone 2 km wide and 4 km long has subsided more than 70 cm (Figs 3*a* and 4*a*). The amount of subsidence decreases towards Lake Asal and the Ardoukoba volcano, and also, although perhaps less rapidly, towards the Ghoubbet. Subsidence is not symmetrical with respect to the geometric axis of the rift, as a smaller rift-in-rift structure to the north is active, with a subsidence of its own of approximately 30 cm (RR in Fig. 4*a*). The general effect of the deformation seen in Fig. 4 is to accentuate the preexisting topography. Both flanks of the inner rift are elevated up to 20 cm over a 1 km width; these zones of elevation correspond roughly to the bands for which the geodetic data indicate contraction. Four km away from the rift axis, both to the north and to the south of it, the gradients of vertical displacement become much smaller. Actually, complementary levelling, although not yet fully processed, shows a broad uplift, some 20 km in wavelength, over which the narrow elevated shoulders and deep subsided central part are superimposed[13].

Field tectonic observations

Detailed tectonic observations were made both on land and from air photographs at a scale of 1/10,000 (refs 15, 16). The recent structures, which seem to be related to the November events, generally follow older structures, with a north-west–south-east trend. Reactivation is clear in recent sediments (such as lacustrine limestones, diatomites and hyaloclastites) and in alluvial plains. Criteria such as freshness of the scarps, offsets in camel and road tracks or creeks have been used. However, it was not possible to find convincing criteria within the recent lava fields; such areas have therefore been left blank on Fig. 3*a*.

Structures fall into two categories: (1) normal faults, with a length from 100 m to several kilometres, and with a maximum throw of 70 cm (Fig. 5*a*); major faults are often associated with smaller antithetic faults. In alluvial zones, normal faults are sometimes paired to form graben structures (Fig. 5*b*) which may in some cases be the surface expression of deeper single faults. (2) Fissures of various types are also encountered: reopened fissures in which detrital filling has subsided (Fig. 5*c*), aligned collapse pits, which are interpreted as the surface expression of underlying fissures below the alluvial cover, and narrow and shallow small grabens that result from a coalescence of the

previous pits. Subaerial faults and fissures that have been reactivated during the events are very numerous and occur in a band of the inner rift which is 3–4 km wide and 8–10 km long (Fig. 3). Part of the deformation is concentrated in the small rift-in-rift structure which is located to the north-east of the inner floor (Figs 3 and 4*b*). The zone of present deformation is offset to the north with respect to the axis of symmetry of the overall rift topography. This suggests a northward migration or jump of the axial rift zone, consistent with the location of the present rift on the northern boundary of the main zone of Plio–Pleistocene lava accumulation and tectonics[7,8,17,18]. There is also a suggestion of *en échelon* rifts, within the inner floor between Ghoubbet and Asal, which is supported by the patterns of seismicity in the eastern part of the Ghoubbet[2,3,9]. This *en échelon* array is reminiscent (although the scale is quite different) of the *en échelon* fissure swarms which occur in the northern neovolcanic zone of Iceland[19,20]. The three smaller *en échelon* fissures that fed the volcanic eruption opened in the northwestern part of the rift, in a region where surface tectonics and seismicity are not otherwise intense.

The vertical throw of faults is largest in the central section of the rift and decreases both towards Lake Asal and towards the Ghoubbet. Transverse tectonic sections were mapped in detail in December 1978 and are shown in Fig. 4*b*. There is a general agreement between the tectonic profiles and the levelling measurements (Fig. 4*a*), particularly for the southern half of the rift: for instance, two major reactivated scarps are crossed between levelling points 33 and 34^{b} (Fig. 3*a*) with a 70 cm downdrop. Tectonic profile 3, which is not far from the levelling route in this neighbourhood, shows a cumulated throw of the same order of magnitude (1 m). However, along the same tectonic profile 3, a discrepancy appears between levelling points 35^{I} and 35^{II}: whereas the area is devoid of identifiable traces of brittle deformation (Fig. 3*a*), the levelling shows a 30 cm elevation difference (Fig. 4*a*). This may be because fresh brittle deformation is more difficult to observe in the lava surfaces which are found in this zone. Similar mismatches are found elsewhere, in a similar context. They may be related either to block tilting or to more distributed deformation of the surface cover in response to deep-seated faulting. Also, it is not certain that levelling and tectonic profiles reflect activity over the same time period.

Episodic rifting along accreting plate boundaries

There are interesting similarities between the observations of seismicity and tectonic deformation presented here and those reported by Björnsson *et al.*[19,20] for a rifting episode on the plate boundary in the northern neovolcanic zone of Iceland. We have already pointed out the common en échelon arrangement of fault and fissure swarms, although in the Icelandic case only one such swarm has been reactivated since the current rifting episode started in 1975. A comparison between Figs 1 and 4 given here and Fig. 6 of the Björnsson *et al.* paper[20] reveals the striking similarity in horizontal and vertical ground movements in both cases. Note particularly the association of axial subsidence and extension with elevated side-shoulders showing evidence of contraction. This similarity between current rifting in Afar and Iceland (despite other differences) suggests that we are witnessing the fundamental mode of deformation at the axis of oceanic ridges. This adds to the already vast set of observations suggesting that there are few qualitative differences between tectonic processes in the axial zone of oceanic ridges and those which can easily be observed directly in Afar and Iceland[21–24].

One should not misinterpret[1] the relationship that may exist between a single event and long term motions occurring at a plate boundary. It is the superimposition of numerous discrete events of this kind both in space and time along the plate boundary that are integrated to give the slow (1 cm yr^{-1}) motion of the plates[17,18,25–27]. Thus, the large extension of ~2 m that occurred between 1973 and 1978 in the Ghoubbet–Asal rift zone does not reflect the increase in distance between the Arabian and African plates in the same time interval.

Elastic rebound and faulting

Perhaps one of the most important results to emerge from the geodetic measurements is that deformation occurred in a coherent pattern, in an area that probably does not exceed a few tens of kilometres in width. The first model which comes to mind to account for these observed deformations is the elastic

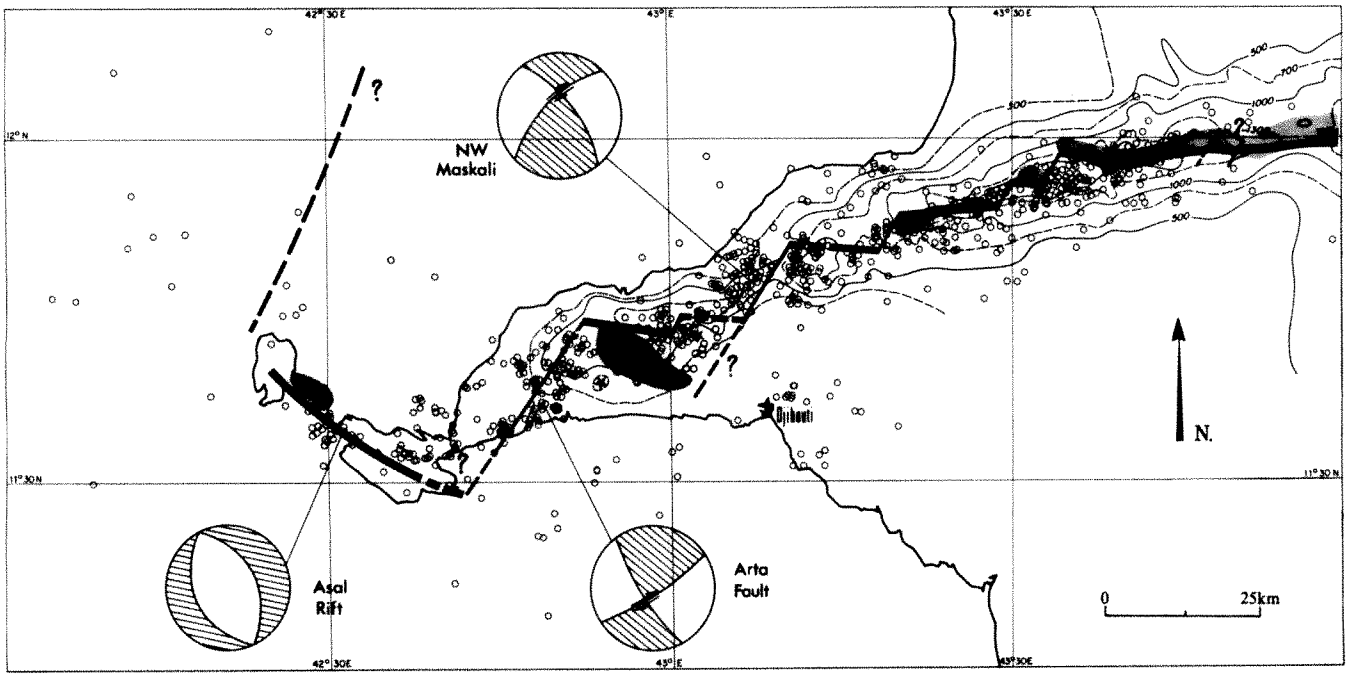
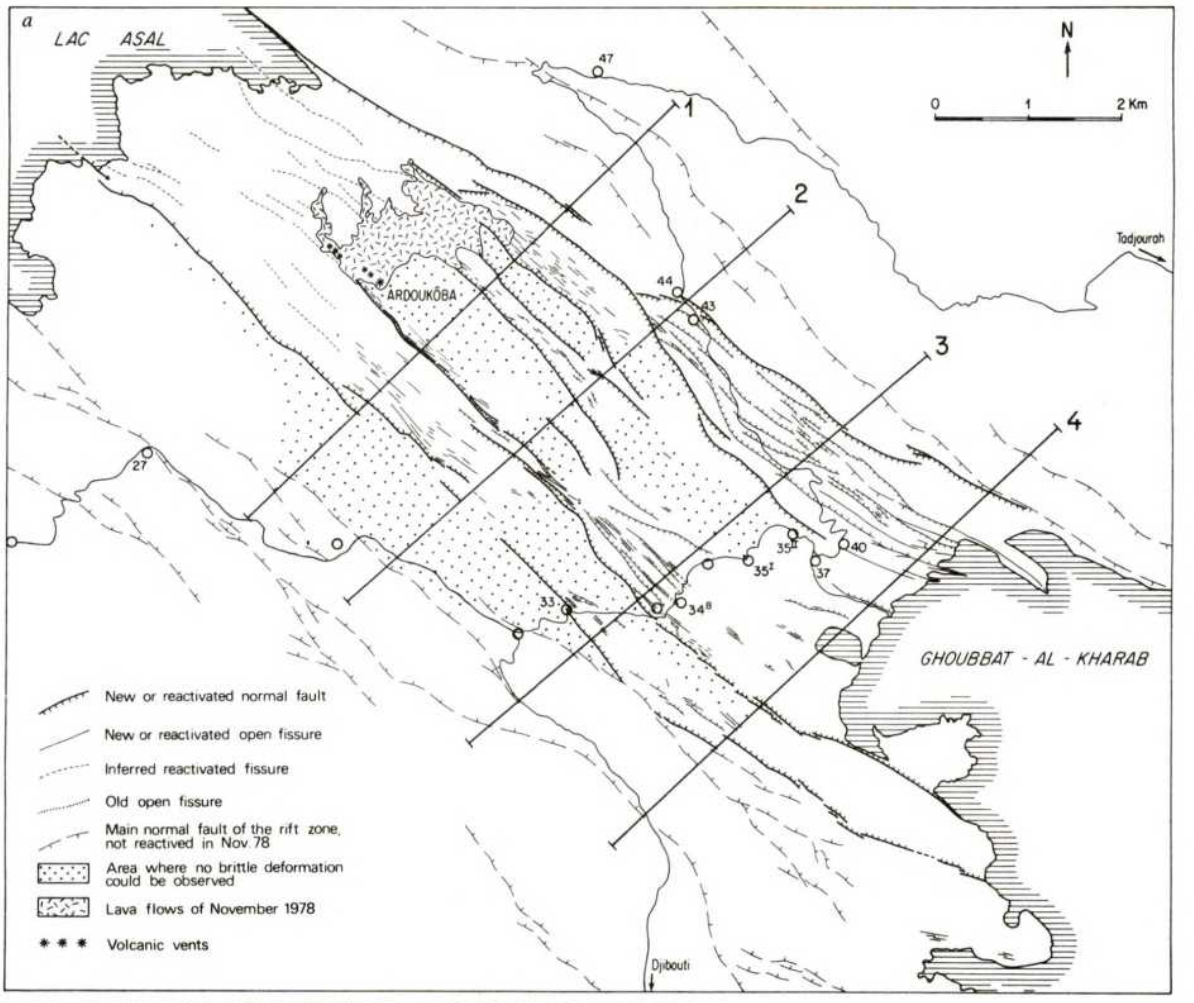


Fig. 2 Detailed bathymetry, earthquakes (○) and composite focal mechanisms for the period 1972–77 (see refs 2, 3, 17). ☆, Submarine volcanoes. Negative axial magnetic anomalies from Fig. 6 are shaded (after ref. 17).



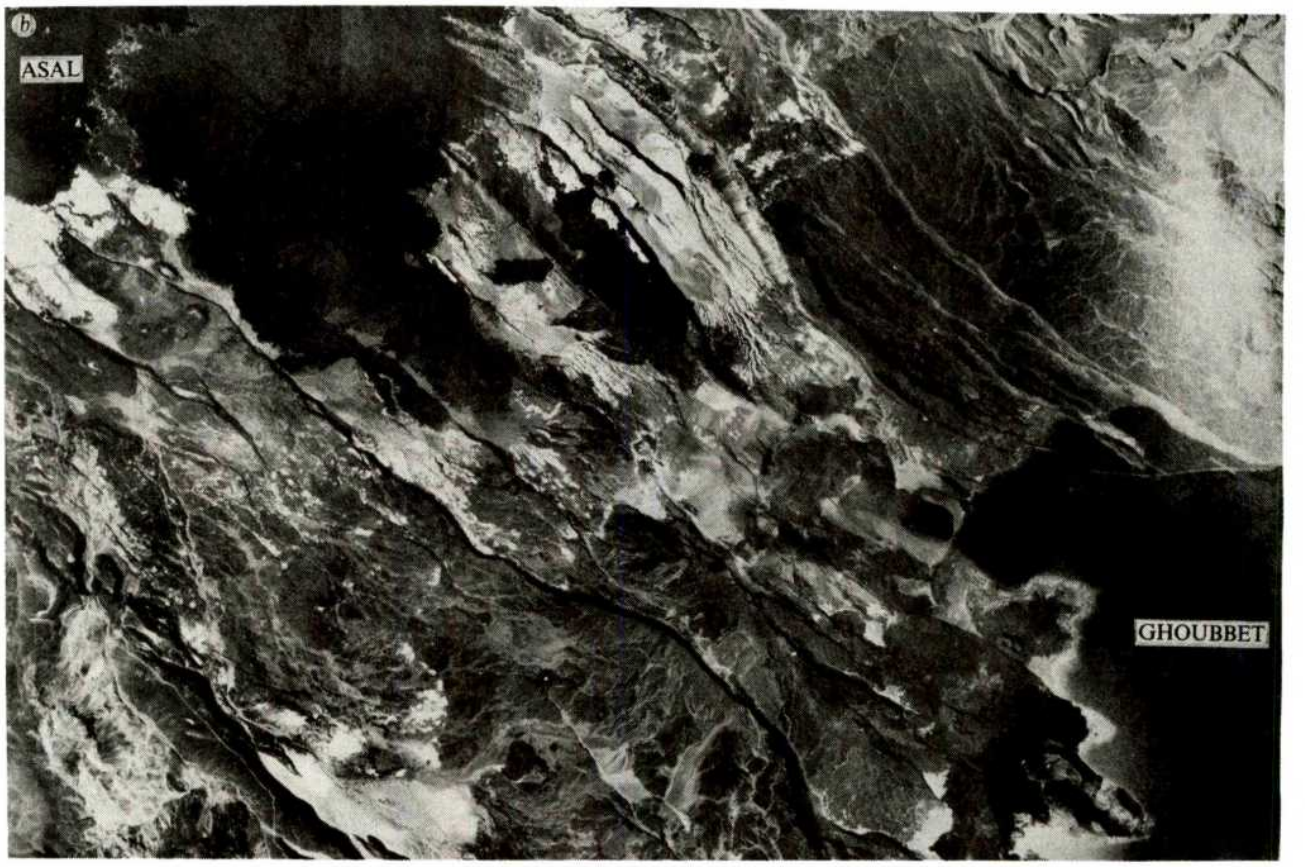


Fig. 3 *a*, Observed new or reactivated tectonic features in the Ghoubbet–Asal rift. Locations of tectonic profiles shown in Fig. 4*b* are given. The levelling route is also shown and several stations are labelled for reference. *b*, Composite air photograph of the same area at the same scale.

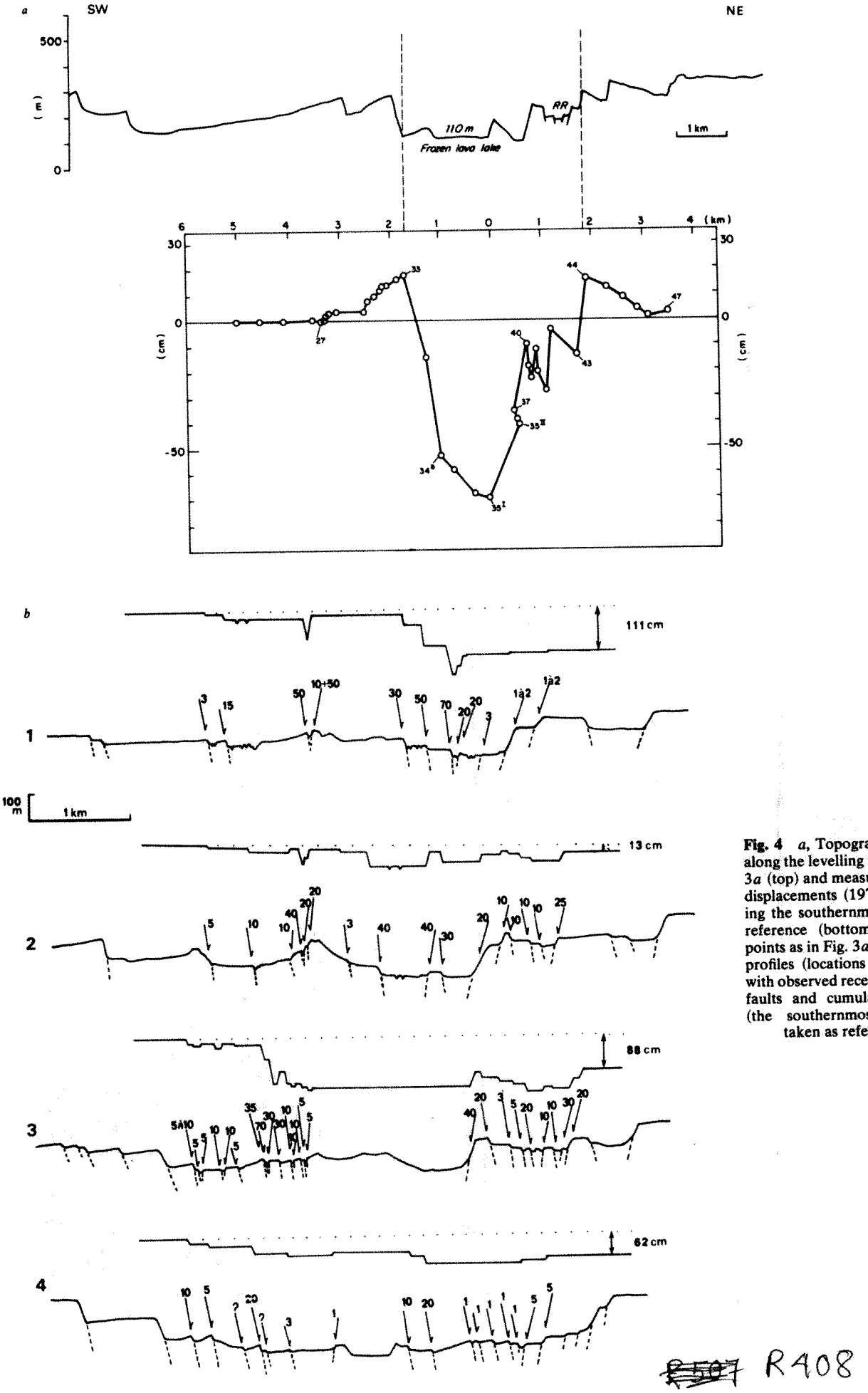


Fig. 4 *a*, Topographic profile along the levelling route of Fig. 3*a* (top) and measured vertical displacements (1973–78), taking the southernmost point as reference (bottom). Labelled points as in Fig. 3*a*. *b*, Tectonic profiles (locations in Fig. 3*a*) with observed recent throws on faults and cumulated throws (the southernmost point is taken as reference).

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rebound model, introduced by Reid[28] for the study of the 1906 San Fransisco earthquake. The model must, of course, be adapted to the case of normal faulting. The global forces that are responsible for plate motions presumably put the whole of Afar under extension with the principal direction being roughly north-east–south-west and energy is stored as elastic deformation builds up. During the seismic events, this energy is released and a zone of tens of kilometres in width is permanently deformed. Mansinha and Smylie[29] give analytical expressions which enable one to compute the displacement fields due to slip on inclined, finite, dip-slip (and strike-slip) faults, which are modelled as Volterra dislocations. Savage and Hastie applied this formula to the case of a fault with both dip- and strike-slip (refs 30, 31). Their results suggest that both vertical and horizontal ground displacements shown in Figs 1 and 3 can reasonably be accounted for by dip-slip faulting (with a throw of more than 1.5 m) on two normal faults with opposite dips, corresponding roughly to the walls of the inner rift. This would especially agree with the observation of both elevated and contracted lateral bands (see, for instance, Fig. 8 of ref. 30). Of course, faulting did not necessarily occur instantaneously on both faults, and these two faults give an integrated picture of the action of many smaller faults during the November 1978 swarm. This deformation is to be added to the deformation that may have taken place in the previous years. Alternatively, the horizontal displacement data can be accounted for by a 3-m opening of two finite vertical fissures, trending north-west–south-east, close to the axis of the inner rift, one in Ardoukoba rift and the second below the Ghoubbet waters where most of the seismicity occurred[14].

We should note at this point that much of the permanent outward tilt of blocks on both sides of the rift may be due to the integration of such deformation episodes and not only to isostatic response[21]. This may help to explain observations of rather large tilts on oceanic ridges.

1978 Events and geodynamical models of Afar

Although various plate tectonic analyses of the Red Sea–Afar-Gulf of Aden area have been published[18,25] it is still apparently very difficult to define plate boundaries within Afar[7,8,17]. On the basis of a new detailed aeromagnetic survey of the Republic of Djibouti (Fig. 6 and refs 7, 8), one of us (V.C.) has proposed that the western termination of the Gulf of Aden and the Gulf of Tadjourah were opened by the westward propagation of a crack in the lithosphere. Magnetic data and rock ages on land are interpreted to show that the crack has been propagating fairly regularly at ~3 cm yr^{-1}. It is suggested that the tip of the crack is now lying in the vicinity of Lake Asal. Bathymetric data and seismicity recorded over the period 1972–78 (refs 2, 3, 9) agree with this suggestion (Fig. 2): in particular, Lake Asal is seen to mark the westward termination of the narrow, well defined band of seismicity which corresponds to the active plate boundary.

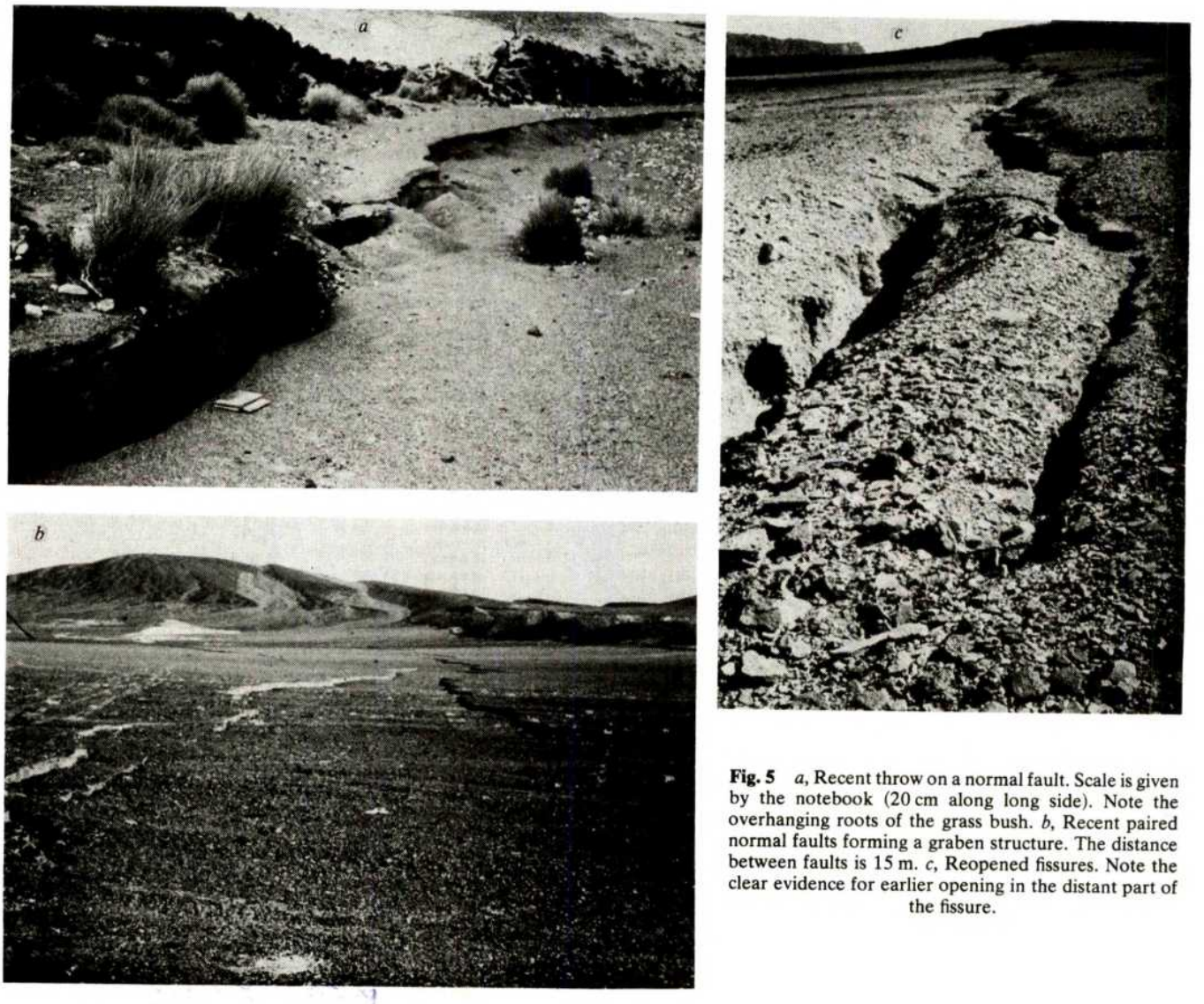


Fig. 5 *a*, Recent throw on a normal fault. Scale is given by the notebook (20 cm along long side). Note the overhanging roots of the grass bush. *b*, Recent paired normal faults forming a graben structure. The distance between faults is 15 m. *c*, Reopened fissures. Note the clear evidence for earlier opening in the distant part of the fissure.

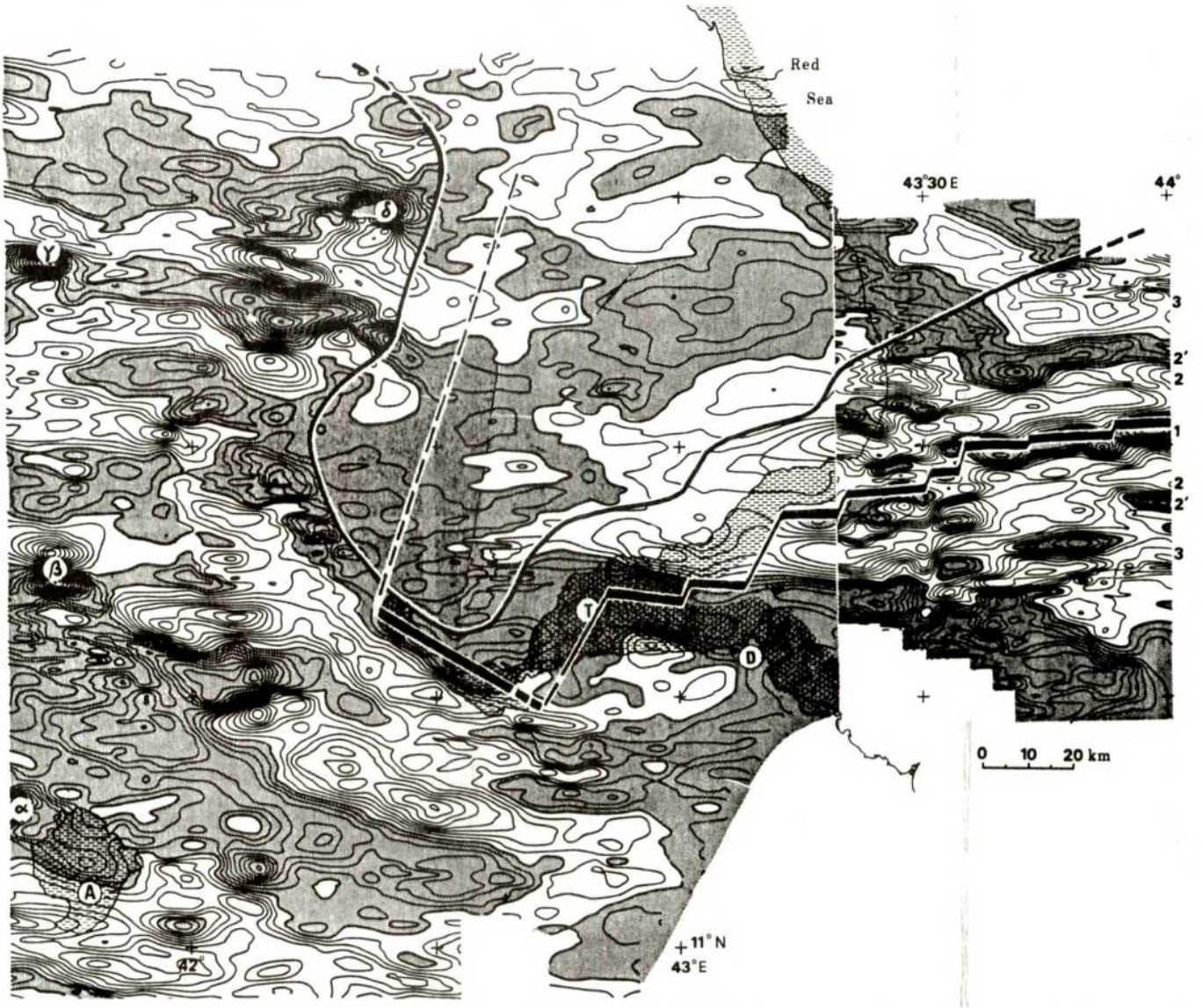


Fig. 6 Aeromagnetic anomaly map of the Republic of Djibouti (after refs 7, 8, 17). The plate boundary is indicated as a sequence of thick (ridge) and thin (transform fault) segments; the Mak'arrasou "transform fault" (refs 4–6) is shown as a dashed line. Also shown as a sinuous line is the boundary between zones of oceanic and more subdued magnetic styles (see ref. 17).

West of Lake Asal, seismicity seems to be more sparse and diffuse.

Thus, the November 1978 events would have started close to the suggested location of the crack tip. It is interesting then that the migration of seismicity away from the crack tip during the swarm and the distribution of the amplitudes of deformation (which are larger in the Ghoubbet than in the immediate surroundings of the volcanic centre) are compatible with the idea that to the west of the crack tip lies a locked area which is more resistant to deformation than the zone along which the plate boundary has already penetrated.

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The eruption of Soufrière volcano, St Vincent April–June 1979

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The Soufrière volcano in St Vincent began to erupt on 13 April 1979 after 10 months of mild premonitory activity. A series of strong vertical explosions between 13 and 26 April generated ash falls, pyroclastic flows and mudflows. From about 3 May onwards basaltic–andesite lava has been accumulating in the summit crater.

ST VINCENT is in the south of the chain of volcanic islands which comprise the Lesser Antilles Island Arc (Fig. 1). The island is 30 km long and the Soufrière volcano constitutes the northernmost third of the island with a diameter of 11 km at sea level. It is a strato-volcano 1,220 m high with an open summit crater 1.6 km in diameter located in the southern part of a 2-km wide somma crater. The summit crater has been occupied by a 1-km wide crater lake throughout most of its history[1–4]. Explosive eruptions occurred in 1718, 1812 and 1902–03; the 1902–03 eruption was preceded by a 14-month period of felt earthquakes, and generated ashfalls, mudflows and pyroclastic flows climaxing on the afternoon of 7 May, 1902, when pyroclastic flows swept down valleys leading to the east and west coasts killing, 1,565 people. The eruption continued intermittently until March 1903 by which time the total volume of uncompacted pyroclastic ejecta is estimated to have been 0.42 km^3 (ref. 5).

In contrast, the eruption of 1971–72[4,6] was aseismic and non-explosive. Between October 1971 and March 1972, 80×10^6 m^3 of basaltic andesite lava were quietly extruded into the summit crater causing the evaporation of 55×10^6 m^3 of water out of an original lake volume of 75×10^6 m^3. During the later part of this eruption seismic activity at the volcano was monitored by three high-gain, short-period seismographs all within 4 km of the crater but no seismic events were recorded other than small surficial events associated with the growth of the lava mass. The number of these events had declined almost to zero by the time that the extrusion of lava ended in March 1972 and they had ceased entirely when the seismograph stations were withdrawn in August 1972.

Activity premonitory to the 1979 eruption

Since August 1972, the nearest seismograph station to the volcano which was in continuous operation was station SVT 19 km to the south. The temperature at the southern edge of the crater lake, which had reached a peak of 81.5 °C in November 1971 was measured twice per month and found to decline steadily until January 1975 when it stabilised between 23 and 26 °C which is 1–4 °C above ambient conditions. The crater lake was inspected about every 3 months. After March 1972, the crater lake and island showed little change other than a gradual erosion of loose material from the island. Fumarolic activity was at first widespread over the island but by 1975 activity was concentrated in a depression of diameter ~20 m in the centre of the island. A bathymetric survey of the crater lake was made in February 1973[7] and surface temperature surveys were made in March 1974 and December 1978. In March 1974, a large yellow stain became visible on the surface of the lake in the south-eastern corner. At this time the temperature around the edge of the lake ranged from 30 to 33 °C, rising to 47 °C close to the lava island. The yellow stain persisted until the start of the 1979 eruption. A similar stain was reported by Senn[8] in 1946 after a series of local earthquakes had been felt in northern St Vincent.

The first signs of abnormal activity which might be related to the present eruption were observed between August and November 1976 when a phreatic eruption of the Soufrière volcano in Guadeloupe 310 km to the north was in progress. Between 11 August and 21 October the water temperature at the southern edge of the lake rose from 23 to 30.5 °C. No other signs of abnormal activity were observed and by 19 January 1977 the temperature had decreased to 25 °C. In February 1978 the temperature began to rise slowly and irregularly, reaching a peak of 31 °C on 21 August 1978. On 2 June 1978 a short-period seismograph linked by radio telemetry to the University of the West Indies in Trinidad was established at Belmont, 9 km south-west of the crater (SVB in Fig. 2) and on 12 June this station began to record events which were tentatively identified as B-type volcanic earthquakes[9]. The events occurred in bursts of up to 20 and their frequency increased throughout June and July reaching a peak on 30 July when 40 events were recognised. Because of the simultaneous rise in lake temperature and increase in seismic activity, the local authorities were advised on 24 August that an abnormal situation might be developing at the volcano. Tilt monitoring stations of the optical tiltmetry type which were first established on the eastern flanks of the volcano in February 1977 at distances of 3 km and 6 km from the crater were re-occupied on 7 March 1978 and again on 17 September 1978. The results showed apparent tilts of order 3–5 μrad, consistent with inflation of the volcano, between March 1977 and September 1978. These were the first optical tiltmetry measurements to be made in St Vincent and the standard error of a single set of measurements was of the order of 4 μrad. The tilt results alone could not therefore be taken as conclusive evidence that the volcano was inflating. Inspection of the crater lake and island showed no significant change but a second telemetered seismograph station established 3 km south-west of the crater on 19 September (SVV on Fig. 2) confirmed that the seismic events originated close to the volcano. From 19 September 1978 to 12 April 1979 these two stations continued to detect B-type events at rates of up to 50 per day but the rate of occurrence showed no tendency to increase or decrease with time. No earthquakes were reported to be felt and only a few of the stronger events were detected by station SVT. During the same period the surface temperature of the lake was measured weekly and fluctuated between 27 and 30 °C up to 11 April 1979 when it was 28 °C. On 24 December 1978 the surface temperature of the lake was measured on one complete circuit of the lake around the island and was found to be a uniform 30 °C except for a hot spot close to the eastern edge of the island where it was 47 °C.

At 07.13 UTC on 12 April a large B-type earthquake was recorded by SVV and SVB and at about the same time continuous tremor became recognisable above the back ground microseismic level. For the following 12 h B-type events continued at

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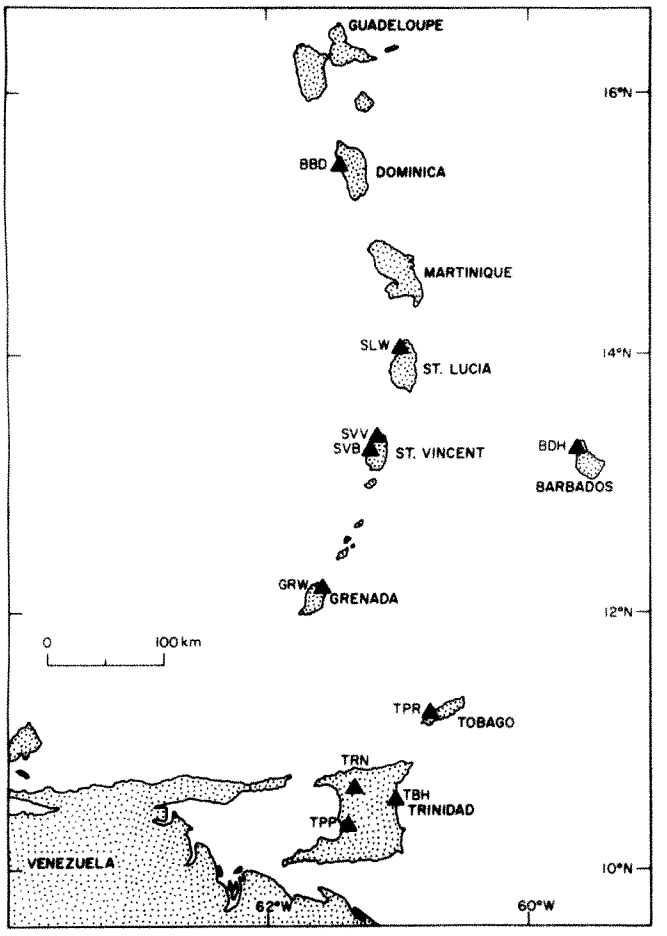


Fig. 1 Regional position of St Vincent in the Eastern Caribbean. The seismograph stations (▲) shown are linked by radio to Trinidad; many other instruments exist in the region.

a mean rate of about one per hour until 19.54 UTC when their frequency began to increase. Between 19.54 and 02.04 UTC on 13 April, 38 events were recorded with generally increasing amplitude and the level of continuous tremor increased steadily. Between 02.04 and 04.30 UTC, 70 events were recorded before continuous tremor saturated both instruments and individual events could no longer be distinguished. At 03.30 UTC (11.30 p.m. on 12 April local time) the government of St Vincent was warned that a highly abnormal situation existed at the volcano, but local residents 6 km east of the crater reported that they had neither seen nor felt anything unusual up to this time. At about 08.00 UTC loud rumbles were heard both east and west of the volcano and shortly afterwards fine ash began to fall at distances of up to 5 km south-east and south-west of the crater. When dawn broke at about 09.30 UTC the volcano was seen to be erupting a column of steam and ash and evacuation of the areas north of the Rabacca and Wallibou rivers (Fig. 2) began immediately. The evacuation was completed smoothly by 20.00 UTC and within the next 24 h about 15,000 people were evacuated from this area.

Early stages of the eruption

Figure 3 summarises the eruptive and associated activity from 12 April to 17 June. From 09.30 to 15.00 UTC on 13 April no one person kept the volcano under continuous observation but from numerous reports we deduce that there was continuous strong steam emission from the crater for the whole of this period, punctuated by powerful vertical explosions which projected ash-laden clouds to heights in excess of 8 km. After the initial outburst further distinct explosive phases were observed at about 10.15 and 15.15 UTC. At 11.20 UTC the eruption column was observed from a commercial aircraft to be at a height of 10 km, 150 km, south-east of the volcano. The column height was then ~8 km and an anvil-shaped cloud extended 80 km to the east. The column was again seen at 12.50 UTC from the same distance and appeared to be unchanged. At 15.00 UTC two of us saw the column from an aircraft approaching St Vincent from the south. When first observed the eruption column and cloud fitted the earlier description, but at 15.15 UTC a dark, cauliflower-shaped cloud was projected upwards above the column and drifted off to the east. At 16.00 UTC we flew low over the eastern and western flanks of the volcano in a light aeroplane and landed briefly at the Rabacca River 7 km south-east of the crater. At this location, fine light-grey volcanic ash was falling lightly but steadily and had formed a layer ~3 mm thick. The aerial inspection showed that a thin layer of ash had been deposited to distances of at least 10 km from the crater. We saw no evidence of pyroclastic flow deposits on the lower flanks, but the uppermost third of the volcano was obscured by falling ash.

After 16.00 UTC the dense steam-and-ash column dispersed although steam continued to pour from the crater. At 19.30 UTC we established an observation post at Belmont (SVB on Fig. 2) and since then the volcano has been kept under continuous visual surveillance. Explosive activity was renewed at 20.05 UTC and further explosive phases began at 21.08 UTC and 23.50 UTC on 13 April and at 01.08, 07.06 and 15.50 UTC on 14 April. The explosive phases varied in duration and intensity but were all broadly similar. Each began with rumbles audible from Belmont followed within 1 min by the emission of a white cloud which rose and expanded rapidly. One to two minutes later the clud became much darker and assumed a distinctive cauliflower shape, rising and expanding rapidly and mushrooming outwards at a height of about 4 km above the crater rim. Ultimate heights of the eruption columns could not easily be estimated from the ground but the eruption clouds generated by the explosions from 21.08 UTC on 13 April onwards were photographed from a NOAA Earth satellite and maximum column heights have been estimated by comparing the temperature at the top of the cloud with standard atmospheric temperatures. By this method the columns from the explosions at 21.08 UTC on 13 April and at 01.08 and 15.50 UTC on 14 April have been estimated to reach heights of 17–18 km. The other eruption columns did not penetrate the meteorological cloud layer and no similar estimates of their heights could be made. The lower part of each cloud drifted to the west in the prevailing trade winds while the upper part drifted to the east in the upper-level westerlies. Transport of the ash-plume resulted in ash fall on Barbados, 180 km to the east on the afternoons of 13 and 14 April. On the western flanks of the volcano air-fall from this series of explosions consisted mainly of fine, light-grey, crystal-rich ash but fresh scoria up to 70 mm in diameter fell on Georgetown, 9 km south-east of the crater. The maximum air-fall thickness at the eastern flank of the volcano 7 km from the crater was 40 mm. All explosive activity originated in the western part of the crater at the approximate location of the island formed in 1971–72.

Pyroclastic flows

During the explosive phase beginning at 21.08 UTC on 13 April we observed a pyroclastic flow which descended the upper part of the Wallibou river valley to the south of the crater. At the Rabacca river crossing 7 km south-east of the crater it was reported that a pyroclastic flow descended part of the way down the Rabacca river valley during the same explosive phase. Further signs that pyroclastic flows were being generated during the phase beginning at 01.08 UTC occurred on 14 April when incandescent material was projected over the crater rim and cascaded down the southern and western flanks. A low-level aerial reconnaissance at dawn (about 10.00 UTC) on 14 April showed a fresh pyroclastic flow deposit in the upper part of the Rabacca valley. The largest pyroclastic flow that we observed descended the 3-km long Larikai valley to the west of the crater at 16.00 UTC on 14 April and continued out to sea for at least 10 km. When inspected on site, 28 h after emplacement, the

deposit at the mouth of the Larikai was 1.5 m thick, ~250 m wide and contained scoria blocks up to 600 mm in diameter in a matrix of ash and lapilli. A pyroclastic flow rich in blocks of scroria and carrying trunks and limbs of trees descended the adjacent Roseau valley at about the same time, to within 100 m of the sea.

Subsequent explosive activity

From 17.00 UTC on 14 April to 20.57 UTC on 17 April the volcano was in a state of mildly explosive activity emitting mainly steam but with occasional cauliflower-shaped, ash-laden clouds rising to heights of 1–2 km above the crater rim. On 17 April the lower tilt-meter sites on the eastern flanks were reoccupied and showed tilts of ~4–6 μrad indicating deflation of the volcano since September 1978. At 20.57 UTC on the same day another explosive phase similar to the previous ones began. Pyroclastic flows were again observed to descend the Larikai, Roseau and Wallibou valleys to the west of the crater but none reached the sea. The height of the eruption column was measured from a NASA aircraft near St Vincent and was estimated to be 18.7 km. Ash fall at Belmont was coarser than in previous explosions. Initially it consisted of lapilli up to 10 mm in diameter grading down to fine ash over a period of ~50 min. Coarse scoria again fell on the eastern flanks as far as Georgetown. All these explosions were accompanied by severe electrical storms in which lightning discharges both within the eruption cloud and from cloud to ground occurred at a rate of more than 1 per s. Lightning strikes were observed at distances of at least 9 km from the crater and caused damage to trees 6 km east of the crater. A striking feature observed during the explosion of 17 April was the development of smooth, funnel-shaped bands of condensation around the eruption column immediately below the cauliflower-shaped eruption cloud.

After the 17 April explosion the volcano returned to a state of intermittent, mild explosive activity, generally declining in intensity but punctuated by two further explosive phases beginning at 10.37 UTC on 22 April and 03.53 UTC on 26 April. During the 22 April explosion a small pyroclastic flow descended the Larikai valley. A few minutes later a dilute pyroclastic flow was formed from ash that fell from a billowing cloud of

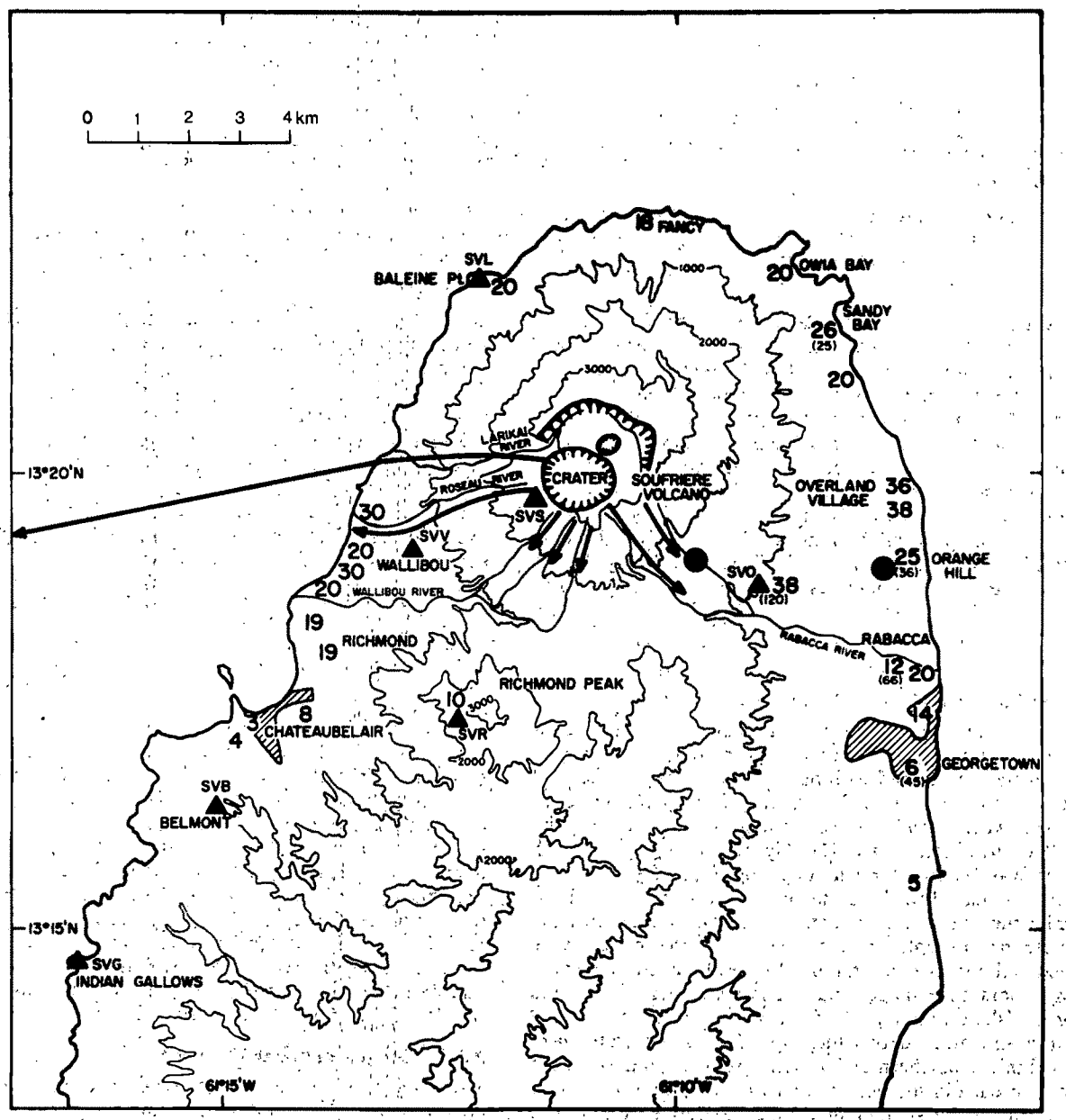


Fig. 2 The northern part of St Vincent island showing the location of the Soufrière volcano, major streams, general topography and two principal towns. The courses of pyroclastic flows known to have taken place in the 1979 eruption are shown as arrows and measurements of ash thicknesses (mm) are given as large numerals. The positions of tiltmeter (●) and seismograph station (▲) are also indicated. Figures in parentheses are maximum bomb dimensions (mm).

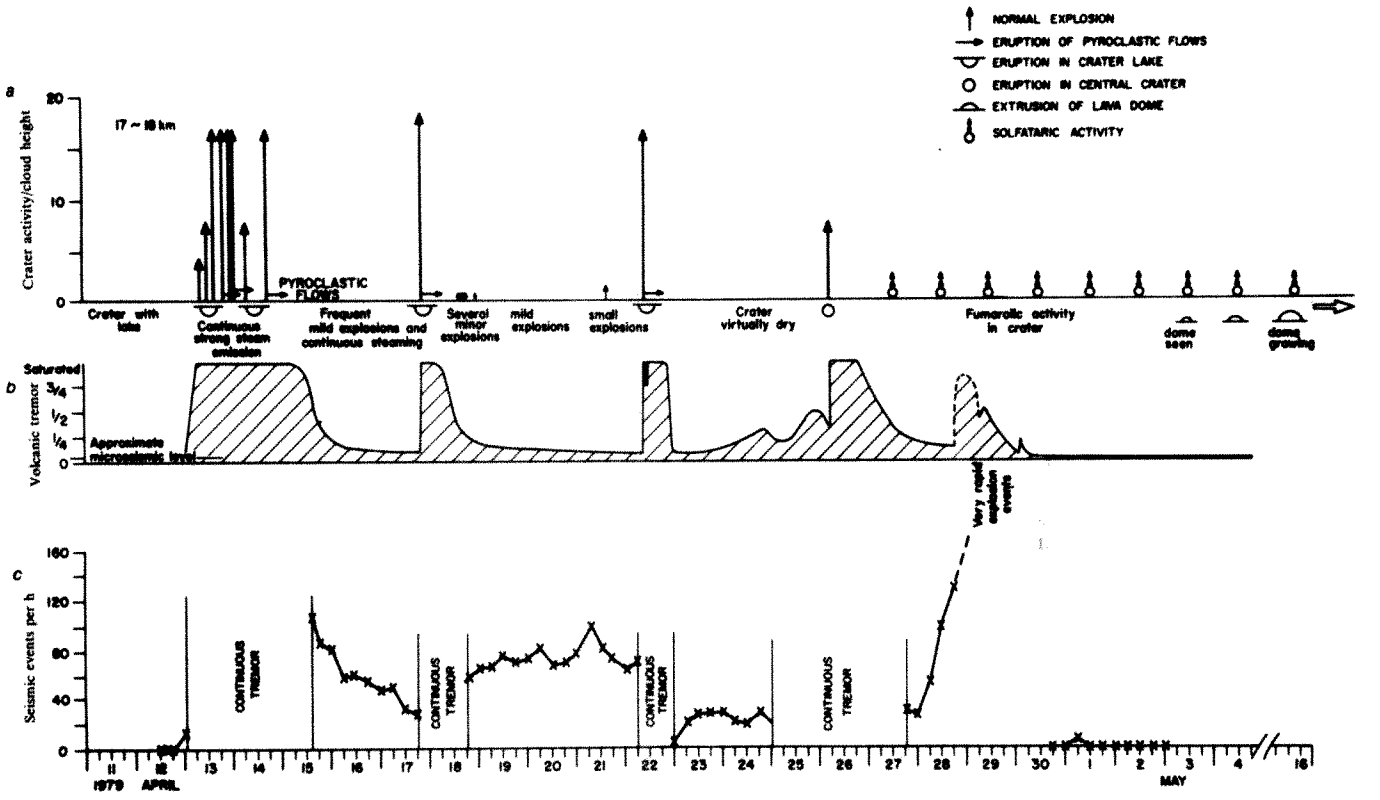


Fig. 3 Chronological summary of the major eruptive events and seismic behaviour of the Soufrière Volcano for the period 11 April–16 May 1979. *a*, The form of activity at the crater; *b*, the approximate level of continuous volcanic tremor; and *c*, the number per hour of discrete seismic events (explosion and B-type earthquakes), when these could be discerned from the continuous tremor.

airborne tephra and this flow, dark grey to black in colour, crossed the coast and spread as far south as Chateaubelair Bay. After this explosion the main axis of ash fall extended east from the crater with maximum total thicknesses along the east coast of the order of 40 mm at Overland Village, thinning to 10 mm at 5 km to the north and south. These figures are lower limits since each ash fall was rapidly eroded by wind and rain soon after deposition. Similarly the pyroclastic flow deposits in river valleys were rapidly reworked by streams and covered by mudflows which began to form on the night of 14–15 April. The final explosion of the series began at 03.53 UTC on 26 April. The height of the eruption column was measured from HMS Gurkha 15 km to the west and was estimated to be 8 km. Wind carried the eruption cloud from this explosion due south and deposited up to 4 mm of ash on Kingstown the capital.

The crater during the eruption

Throughout the explosive period frequent low-level reconnaissance flights were made over the volcano but the continuous steam emission combined with persistent low-level cloud prevented us from seeing into the crater until 17 April at about 13.00 UTC, when a brief view was obtained of the interior of the crater. The 1971 island was still largely intact except to the north where it was breached by a hole approximately 100 m in diameter connected to the crater lake. It was not possible to determine the extent or depth of the crater lake. On 21 April the first clear photographs of the interior of the crater were obtained. About one-quarter of the 1971 island remained, forming a horseshoe shape around a steaming vent. South and east of the island remnant, the floor of the crater was covered with mud and other debris but a portion of the crater lake remained in the northern and western parts, connected to the vent by a breach in the northern part of the island. Persistent heavy cloud at the summit then made aerial reconnaissance extremely difficult but on 30 April conditions improved and it was possible to see that only a fragment of the island remained. Most of the floor was covered with mud around a strongly steaming central depression. On 3 May the presence of a small dome-shaped object in the eastern crater rim was reported to be steaming vigorously. Aerial reconnaissance on 7 May confirmed the presence of a fresh, black, dome-shaped mass of lava ~200 m in diameter rising to ~30 m above the crater floor. The crater itself was infilled with debris to above the level of the pre-eruption lake surface and was apparently dry except for a small pond abutting the northern edge of the new lava mass. Strong steam emission was taking place at the western edge of the lava and at several other places on the crater floor. The lava itself was draped with sediments which eroded rapidly over the next two days. Further aerial inspections were on 8, 9 and 11 May during which time the lava mass increased in size by about 40%. Between 14 and 16 May the lava mass was surveyed from the crater rim and from the crater floor. At this time it was approximately elliptical in plan with major axes 400 m from north to south and 300 m from east to west. Its sides sloped upwards at an angle of about 45° to a flat top with a slight depression in the centre. A small crescent-shaped pond abutted the lava on the north side while to the south-west and south-east two relics of the 1971 island protruded through the new crater floor. During the next month the lava continued to grow, mainly by lateral spreading over the crater floor, at a rate estimated to be $0.5–1 \times 10^6$ m^3 d^{-1}. Growth was most rapid to the north and north-east and least rapid to the south and south-east where the remants of the 1971 lava restricted lateral spreading. The depression on the top of the lava gradually disappeared to be replaced by a slight rise from which several low ridges radiated. On 18 June the diameter of the lava was 715 m from east to west and about 700 m from north to south. Its height was estimated to be 110 ± 10 m and its total volume of order 25×10^6 m^3.

Seismological observations

Three short-period vertical-component seismographs were in operation in St Vincent at the beginning of the eruption. Two of these (SVV and SVB in Fig. 2) were linked by radio telemetry to Trinidad where the signals were displayed visually on drum recorders and simultaneously recorded on magnetic tape for later analysis. The third station (SVT) operated with a standard

Table 1 Major oxide analyses of Soufrière samples

Component	Grey glass*	Dark glass*	Scoria block†	Composite ash fall‡	Lava flow§
SiO_2	65.9	57.9	54.9	55.5	55.8
Al_2O_3	13.7	14.3	18.7	19.2	18.4
FeO‖	6.77	9.38	8.23	7.43	8.58
MgO	1.23	2.86	4.08	3.44	4.05
CaO	4.05	6.34	8.94	8.70	9.11
Na_2O	3.34	4.02	3.35	3.43	3.32
K_2O	1.56	1.11	0.56	0.58	0.56
TiO_2	1.08	1.64	1.01	0.97	1.05
P_2O_5	–	–	0.12	0.12	0.11
Total	97.63	97.55	99.89	99.37	100.98

The modal analysis of air-fall ash collected at Grantley Adams Airport, Barbados, 14 April 1979 gave: crystals, 43.5%; grey glass, 33.7%; dark glass, 8.7%; lithics, 13.3%; carbonate and sulphate, <1.0.

* Barbados ash fall, 14 April 1979.

† Larikai pyroclastic flow, 14 April 1979.

‡ Belmont, 17 April 1979.

§ New analysis of sample from lava flow, 13 December 1971.

‖ Total iron oxides reported as FeO.

photographic recorder in St Vincent. On 14 April, a second magnetic tape recorder and visual monitor were set up at Belmont. Initially signals were recorded from SVV, SVB and from SLW, 80 km to the north in St Lucia, BDH, 170 km to the east in Barbados, and BBD, 250 km to the north in Dominica. Other seismograph stations, monitored in Trinidad, were in Grenada, 130 km south of St Vincent and in Trinidad and Tobago. Throughout the eruption the larger network recorded only regional seismic activity at a level known to be normal for this region. In the vicinity of St Vincent the network has a detection capability for earthquakes down to about magnitude (m_b) 2.5, and no earthquake above this magnitude originated within 100 km of the Soufrière throughout the eruption. Between 15 and 21 April four more radio-linked stations were added to the network in St Vincent. On 28 April SVT was linked in to the system and on 12 May a station was established on the crater rim. The final station configuration is shown in Fig. 2. From the time that stations SVV and SVB became saturated by continuous tremor at 04.30 UTC on 13 April they remained saturated until about 13.00 UTC on 15 April (for the entire early period of explosive activity). At about this time the level of continuous tremor had declined sufficiently for individual events to be recognisable, and since then the numbers of events have fluctuated as shown in Fig. 3. A more thorough analysis of these events will be discussed elsewhere but, in the terminology of Minakami[9], they are of two types—explosion-type earthquakes and B-type earthquakes. The relative time control between the stations of the network is $\sim\pm0.02$ s but the continuous background tremor throughout the eruption has made it difficult to identify first onsets to better than 0.1 s. The pattern of arrival times at stations in St Vincent makes it clear that all the events originated within 1 km of the crater, but the emergent nature of the onsets and the station distribution (particularly the lack of a station very close to the crater rim) make it impossible to estimate focal depths by conventional first-arrival techniques to a precision better than ±5 km. A reasonably good correlation was established between some of the larger explosion-type events and actual explosions visible at the crater. Both types of event were propagated with very low phase velocities and attenuated rapidly with distance which suggests they were of extremely shallow origin.

Perhaps the most interesting seismological feature of the eruption has been the continuous tremor. Apart from the initial 57-h period of very strong tremor, there have been several subsequent periods of tremor (Fig. 3). Each major explosive outburst has been accompanied by strong tremor, but no clear premonitory pattern emerged by which we could predict the onset of individual explosions by more than a few minutes. No earthquakes have been reported to be felt during the eruption, but several people who were within 10 km of the volcano on the morning of 13 April reported feeling continuous tremor, and the tremor accompanying the 17 April explosion was reported to be felt at Georgetown.

Petrography and analysis of ejecta

Thin sections of fresh material from bombs and pyroclastic flow deposits show that plagioclase phenocrysts with oscillatory-zoned rims and shadow-zoned cores are the main crystalline phases. Some crystals, the largest of which are 10–12 mm long, contain gas bubbles and inclusions of olivine and glass. Other phenocrysts are pale yellowish-green clinopyroxene with rare and almost colourless orthopyroxene. Accessory amounts of subrounded olivine as well as euhedral amphibole and disseminated magnetite are also present. Fine-grained gabbroic cumulate inclusions 5–20 mm in diameter are common in many of the blocks and bombs. The matrix is glassy to microcrystalline. Tephra collected at Grantley Adams Airport, Barbados, on 14 April 1979 were analysed at the University of Rhode Island and the glasses from the same ashes were analysed by electron microprobe. The results are shown in Table 1. Whole-rock analyses of scoria collected from the 14 April Larikai pyroclast flow and of a composite scoria and ash fall at Belmont on 17 April were carried out at the Smithsonian Institution by electron microprobe. A sample from the 1971 lava was also analysed on the same instrument and all the results are shown in Table 1. The tephra produced in the eruption was found to contain two glass populations of dacitic and andesitic composition. The occurrence of banded scoria blocks in the new air-fall deposits on St Vincent confirm that the 1979 event was a mixed-magma eruption.

Comments

A full assessment of the size and significance of this eruption, the most intensively ever monitored in the Lesser Antilles, cannot be made until all the data have been analysed or indeed until the eruption comes to a definite end. Since 13 April, samples have been collected of the ejecta from each major explosion and the extent and thickness of these deposits have been constantly assessed. After each explosion the air-fall deposits other than those on the uppermost slopes had almost completely vanished within 48 h and the deposits on the volcano itself were partially reworked by mudflows during the same period. These observations illustrate the need for rapid sampling and also suggest that eruptions of the Soufrière which are of great significance from the point of view of public safety may leave little or no permanent stratigraphic record. Several tens of thousands of volcanic earthquakes, as well as many hours of continuous tremor, have been recorded on magnetic tape and most of the individual explosion since 15.15 UTC on 13 April have been extensively photographed, and the explosion of 17 April was particularly well recorded. The air-fall ash thicknesses of Fig. 2 are generally 1–2 orders of magnitude less than the thicknesses reported from the same locations after the explosions of 7–8 May 1902. Similarly the pyroclastic flows seem to have travelled much shorter distances than in 1902 when pyroclastic flows frequently reached the sea both east and west of the crater. Judging by these rough criteria we estimate that the total volume of pyroclastic ejecta in 1979 has been one or two orders of magnitude less than in 1902.

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Plio–Pleistocene environments at Gadeb prehistoric site, Ethiopia

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Widespread artefact-bearing gravels overlie Pliocene diatomites in the plain of Gadeb (7°02'–7°13'N, 39°15'–39°28'E) at 2,300 m in east-central Ethiopia. The diatomites are from 2.7 to 2.35 Myr old, and the bulk of the Developed Oldowan and Acheulian tools are between 1.5 and (?) 0.7 Myr old. Drying out of the Pliocene lake, deposition of the gravels, and erosion of the Webi Shebele gorge reflect the interplay of volcano-tectonic and climatic events, as does the biostratigraphy of the Pliocene diatomites.

A PROBLEM for prehistorians and earth scientists working in East Africa is how to distinguish depositional changes in lakes and river valleys which stem from climatic oscillations from those related to volcanic and tectonic activity[1–4]. A further difficulty near the African Rift Valleys is that much of the older field evidence has been faulted, tilted, removed by erosion, or buried so that conflicting interpretations of the age and significance of Plio–Pleistocene deposits in East Africa make it difficult to reconstruct prehistoric environments during this critical period in hominid evolution with appropriate precision[5–8]. Accordingly there is a real need to seek out areas in Africa in which complete sequences of late Cenozoic deposits have been preserved from erosion and tectonic disruption, and in which evolutionary changes in the plants and animals may be related to prehistoric cultural changes, set within a radiometrically dated time scale. Recently mapped and excavated fluvio–lacustrine deposits at 2,300 m on the plain of Gadeb, near the headwaters of the Webi Shebele River, in the volcanic uplands of east-central Ethiopia (Fig. 1), seem to fulfil these prerequisites, and may well become a stratigraphic datum for the rest of East Africa.

At Gadeb, artefact-bearing fluviatile sands and gravels overlie Pliocene lacustrine diatomites and clays (Fig. 2). The fluviatile sediments occupy erosional channels, and postdate a 2.35 ± 0.2 Myr old welded tuff which overlies the lake deposits. A marked erosional unconformity separates the lacustrine and fluviatile series.

The stone implements associated with the fluviatile sediments are manufactured mostly of local basalt and welded tuff, and belong to the Developed Oldowan and Acheulian traditions[9,10]. The occasional obsidian tools must, on present knowledge, have come from sources in the Rift some 100 km away (W. H. Morton, personal communication). Most, but not all, of the artefact-bearing sediments postdate a 1.5 Myr pumice deposit, and may be older than 0.7 Myr if the initial result from a palaeomagnetically reversed sample above the occupation floors is confirmed by the scores of other samples now being analysed (Fig. 3) (A. Cox and L. Lee, unpublished data). Details of the K–Ar and palaeomagnetic dating techniques will be given elsewhere.

Pliocene Lake Gadeb came into being as a result of one or more lava flows which dammed back the east-flowing drainage of the ancestral Webi (Figs 1 and 2). The issue of hominid occupation of the Gadeb upland plains is related to the problem of when and why Lake Gadeb finally dried out, which is in turn associated with major tectonic and climatic events in Ethiopia. In this article we interpret the regional stratigraphy and outline the environmental changes at Gadeb during the past 3 Myr. Later papers will deal in detail with the geochronology, prehistory, palaeo-ecology and sedimentology, and are part of a

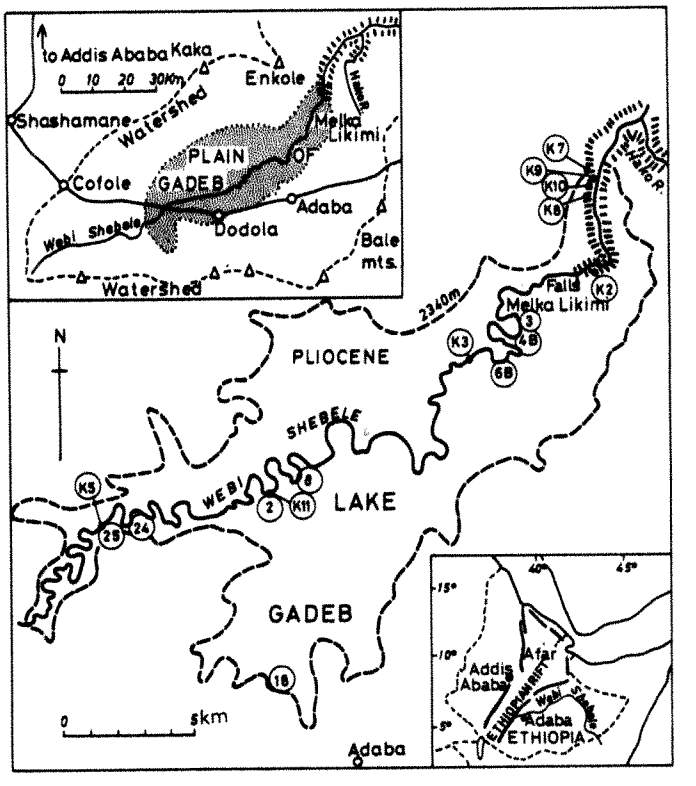


Fig. 1 Location of Pliocene Lake Gadeb, of selected stratigraphic sections (2, 3, and so on), and of K–Ar sample sites (K2 and so on). Triangles along watershed in top left inset map are major volcanoes, often glaciated. Deduced extent of former lake conservative.

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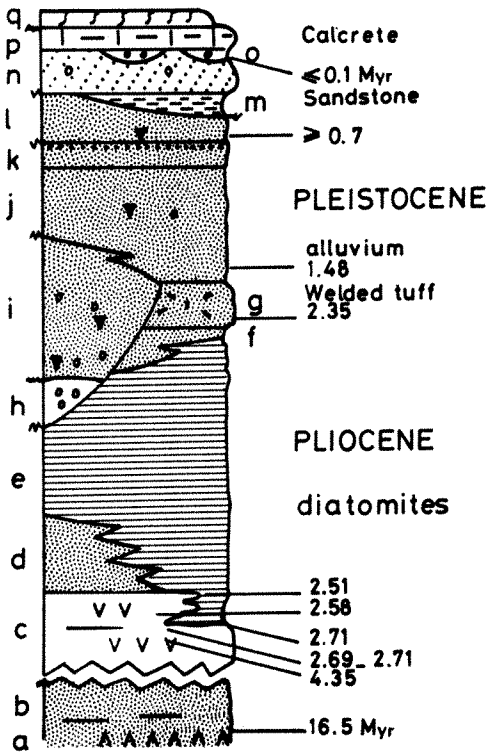


Fig. 2 Generalised Plio–Pleistocene stratigraphic sequence at Gadeb plain, Ethiopia. Ages are based on K–Ar dates (16.5–1.48 Myr), (?) reversed palaeomagnetism (≥0.7 Myr), and presence of Middle Stone Age artefacts (≤0.1 Myr). Letters denote lithological units: a, trachyte; b, tuff; c, basalt; d, feldspathic sands; e, diatomite; f, feldspathic/pumiceous sands; g, welded tuff; h, pumice mudflow; i, sands and fine gravels; j, gravelly sands; k, tuff and palaeosol; l, pumice sand; m, laminated pumice silts; n, pumiceous conglomerate sandstone; o, fine basalt gravels; p, calcrete caprock; q, modern clay soil. Inverted triangles denote developed Oldowan and Acheulian stone tools.

multi-disciplinary study of the Plio–Pleistocene environments and prehistory of east-central Ethiopia.

Geological setting

Flanked by once glaciated[11,12] and now wooded Tertiary volcanoes which rise to over 4,000 m, Gadeb plain is now drained by the Webi Shebele, one of the great rivers of East Africa (Fig. 1). The Webi rises some 250 km south of Addis Ababa, meanders eastwards across the present plain (and former floor of Pliocene Lake Gadeb), and descends suddenly over several high falls into its gorge, initially 300 m deep. Horizontal beds of ash, tuff and trachyte capped by several resistant basalt flows crop out in the sides of the gorge, which is entrenched into an older valley with gently sloping sides. Basalts from five sections along and upstream of the gorge have yielded middle to late Pliocene ages, and a single trachyte sample from ~130 m below the shoulder of the gorge has a K–Ar age of 16.5 ± 0.2 Myr, indicating a Miocene to Pliocene age for the volcanic rocks beneath the Gadeb lake deposits.

The oldest diatomites exposed along the edge of the gorge overlie basalts dated to 4.35 Myr, 2.69–2.71 Myr, and 2.51 Myr (Fig. 2). At one place a 2.71 Myr flow overlies baked and contorted diatomites. We conclude that Lake Gadeb had formed by 2.71 Myr, and that the basaltic eruptions responsible for creating the original lava dam persisted for some time after the lake had come into being. The nearby shield volcanoes of the Bale Mountains are a possible source of these lavas.

During the Pliocene uplift and faulting associated with the creation of the Ethiopian Rift, tuffs and ignimbrites were laid down over the floor and margins of the Rift[13,14]. These pyroclastic deposits thin out away from the Rift, and only a single thin Pliocene welded tuff dated to 2.35 Myr extends as far as Adaba village near the foothills of the Bale Mountains (Fig. 1). By this stage, Pliocene Lake Gadeb had dwindled to a series of small lakes and swamps situated near the centre of the basin.

Pliocene Lake Gadeb

The lacustrine deposits comprise a very pure basal diatomite (3×10^3 valves per mg), passing progressively upwards into initially diatomaceous clays and silts. Intercalated sandy horizons within the diatomite sequence indicate short-lived lake regressions. Abundant interstratified ash bands reflect intermittent volcanic activity within the Rift Valley throughout the existence of Lake Gadeb. The approximate extent of the former lake may be deduced from deposits which attain elevations up to 2,340 m (Fig. 1). The lake had a minimum surface area of 155 km^2 and was at least 40 m deep.

To reconstruct the ecological history of the lake, the diatom assemblages in four closely correlated reference sections were analysed (Figs 1 and 4). Taken together, these four sections encompass the entire lacustrine sequence, a total of over 31 m

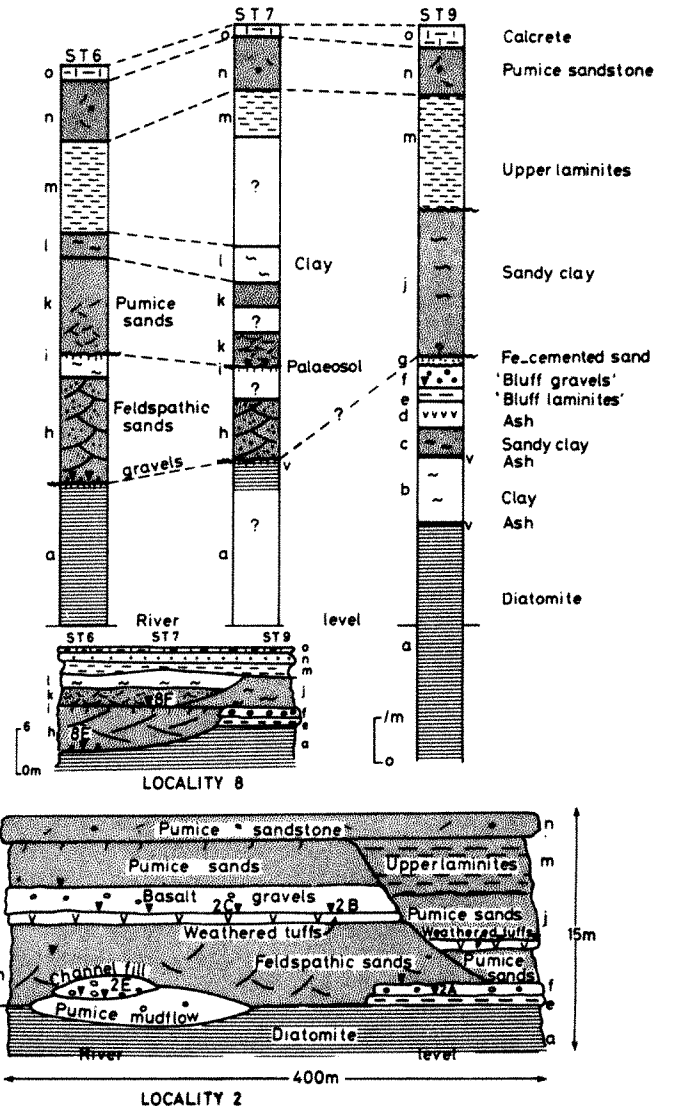


Fig. 3 Pleistocene fluviatile sequence at localities 2 and 8, Gadeb plain, Ethiopia. Inverted triangles denote artefact concentrations (Developed Oldowan and Acheulian), ST is a geological step trench, and zig-zags are unconformities. 2E, 8E and 8F are major archaeological excavations.

(Fig. 4). Analysis of 215 diatomite samples reveals a rich flora (345 taxa) with very diverse assemblages (Fig. 4). The abundance of extinct species makes a detailed ecological interpretation difficult, but it is possible to deduce the ecological influence of certain factors. The relative proportions of planktonic and littoral species allow us to distinguish fluctuations in lake level. On this basis, four major stages, separated by minor regressions, may be recognised (stages I to IV, Fig. 4). Each stage is defined by a set of characteristic species. Despite slight increases in salinity during regressions, the lake was always oligohaline. By comparison with the lakes of East Africa[15], Lake Gadeb was always of low to moderate alkalinity (2–10 mequiv. l^{-1}). Deposition of airfall or reworked ash was also influential, because during stages I and II there is a noticeable peak in the abundance of the ecologically little-known *Stephanodiscus minutus* and *S. tenuissima* immediately above each ash band (Fig. 4). Previous workers have noted that *Melosira* spp. require abundant dissolved silica[15,16], and that an influx of volcanic ash can trigger their sudden proliferation[17].

The increase in detrital material towards the top of the lake sequence reflects a fall in lake level, also evident in the diatom flora, followed by progressive drying out of the lake during stage IV. Nevertheless, the silts and sands that accumulated during the final regression of Pliocene Lake Gadeb are sometimes interrupted by thin diatomaceous horizons laid down during a brief return to more swampy conditions. These discontinuous lenses cannot be correlated from one section to the next, and only occur in a few sections near the centre of the basin. We conclude that these deposits do not point to a renewed transgression of the original lake, but in fact correspond to its fragmentation into a series of small swamps, the position of which varied with the changing locus of drainage. These ponds persisted during the ensuing phases of river erosion and deposition contemporary with the arrival of tool-bearing hominids.

Arrival of tool-bearing hominids

As the level of Lake Gadeb fell, alluvial gravels and sands were distributed across the plain by rivers flowing down from the adjacent uplands. Abundant stone flakes, chopping tools, bifaces and cleavers, found in association with broken animal bones, indicate that tool-making groups were occupying the central parts of the plain at this time[9]. The absence of any stone tools or waste flakes in alluvium beneath and immediately above the intact lake deposits at section 18 (Fig. 5), suggests that there were few, if any, tool-making hominids here at this time. Concentration of slightly abraded implements on the floors of shallow channels formed during the final stages of the dwindling lake (Fig. 3) suggests that the hominids probably camped alongside these channels, hunting the animals that lived in, or came down to drink at, the shallow lake remnants near the centre of the basin.

Plio–Pleistocene river deposits

The fluviatile sediments that overlie the diatomites are highly variable in particle size and composition, ranging from fine pumice silts to feldspathic sands to basalt gravels. Nevertheless, after allowing for facies changes, a progressive change is evident in the depositional pattern with time.

At localities 2 and 8, the youngest diatomites are capped by an indurated and very distinctive layer of horizontally bedded tuffaceous mudstones (the 'bluff laminites' of Fig. 3). Unconsolidated ashes and clays sometimes underlie the mudstone band, which is in turn overlain by a horizontal bed of coarse basalt gravel (the 'bluff gravels' of Fig. 3), often iron-cemented and containing bone chips and stone tools at locality 2 (excavation 2A, Fig. 3). These tools are the oldest discovered at Gadeb so far, and are separated from younger tool-bearing gravels by a sharp erosional break at the base of bed h (8E Fig. 3).

Above the unconformity at locality 8 are tool-bearing basaltic gravels and feldspathic sands which are capped by a palaeosol on which was found a hippo butchery site (8F Fig. 3), well preserved

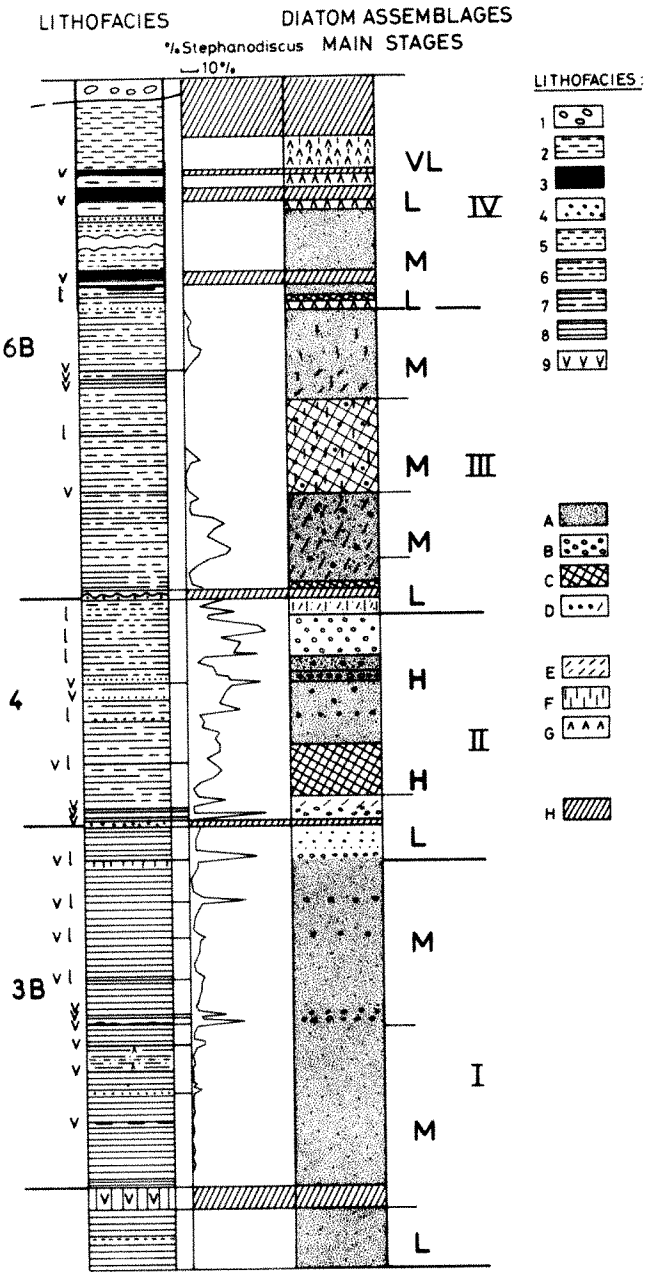


Fig. 4 Pliocene lacustrine sequence at Melka Likimi sites 3B, 4 and 6B, Gadeb plain, Ethiopia. Key to lithofacies: 1, gravels; 2, clay; 3, ash; 4, sand (reworked ash, pumice or feldspars); 5, silt; 6, silty diatomite; 7, clayey diatomite; 8, pure diatomite; 9, basalt; v, volcanic ash; l, limonite band. Key to dominant diatom assemblages: planktonic and tychoplanktonic spp.: A, *Fragilaria* spp.; B, *Stephanodiscus* spp.; C, *Melosira* spp.; D, *Cyclotella* spp.; benthic and epiphytic spp.: E, *Synedra* and *Achnanthes* spp.; F, *Navicula* spp.; G, *Nitzschia, Cymbella, Epithemia, Rhopalodia* and *Pinnularia* spp., H, no diatoms. Lake level: VL, very low; L, low; M, intermediate; H, high.

beneath a thick layer of finely cross-bedded pumice sands. Several weathered and reworked clayey tuffs overlie these sands and are in turn covered by a wedge of finely laminated pumice silts (the 'upper laminites' of Fig. 3) and by a resistant ledge of pumiceous sandstone conglomerate (Fig. 3).

At locality 2 we also have the sandstone caprock and the eroded bluff gravels. Beneath the upper laminites (m) is a thick channel-fill deposit of loose pumice sand (j) similar to that at locality 8. Unconformably beneath the pumice fill are pumice sands and tool-bearing gravels (Fig. 3, locality 2, 2B, 2C) and a

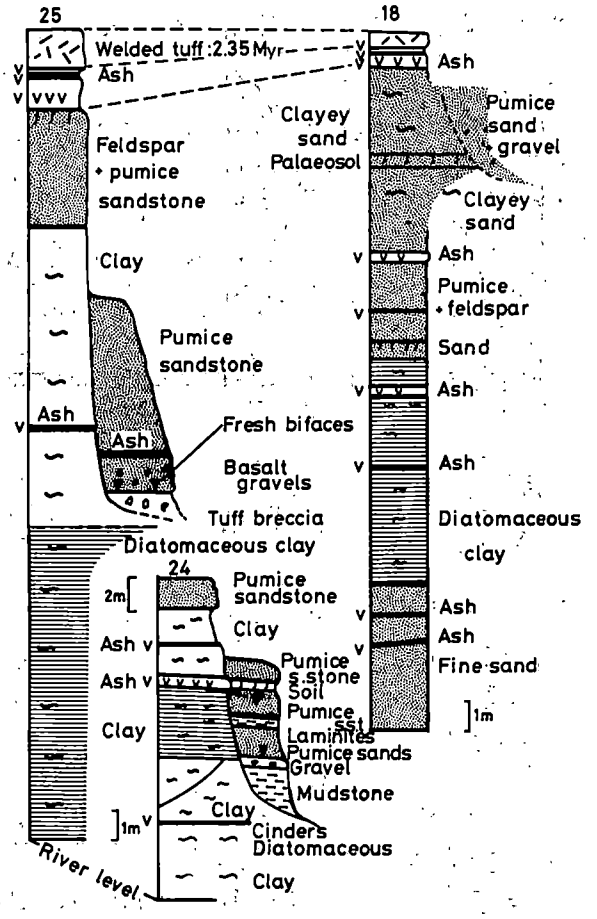


Fig. 5 Plio–Pleistocene depositional sequence at localities 18, 24 and 25, Gadeb plain, Ethiopia. The mineralogy and refractive indices of the three tephra units beneath the welded tuff at localities 18 and 24 show that they are identical at both localities. Inverted triangles denote prehistoric artefacts.

sterile weathered tuff overlying gravelly feldspathic sands (h) similar in appearance to those at locality 8. A conspicuous channel-fill within these sands contains abundant bones and artefacts (Fig. 3, locality 2, 2A), and is in turn underlain by a pumice mudflow deposit dated to 1.48 Myr (Fig. 3).

From the base to the top, therefore, the fluvial depositional sequence at 2 and 8 begins with basaltic gravels and feldspathic sands, then alternates between feldspathic and pumiceous sediments, and culminates in massive deposits of pumice. These changes reflect the incidence of major eruptive phases in the Rift, the nearest source of pyroclastic deposits. Episodes of widespread deposition of basalt gravels at Gadeb rather than of ash imply eruptive quiescence in the Rift at least as much as they imply effective erosion in the Bale Mountains, so that it would be unwise to attempt a purely climatic interpretation of these gravels.

Figure 5 shows how the pumice sandstones and basaltic gravels containing very fresh welded tuff bifaces (section 25) are banked up against the littoral diatomaceous clays and overlying clays, sands and ashes. The 2.35 Myr welded tuff at sections 24 and 25 was breached by vertical erosion before the deposition of the gravels, and before the arrival of the hominids who quarried and flaked the welded tuff.

Erosion of the gorge

Above the head of the gorge the Webi is entrenched some 20 m into the Plio–Pleistocene lake and river deposits described above. Below the falls there is a thin scatter of basalt pebbles and rolled bifaces for 6 km along the southern shoulder of the gorge until the junction with the Hako River[9] (Fig. 1). Scattered diatomite remnants crop out along both flanks of the gorge, but only above the falls do thick artefact-bearing gravels rest directly and discordantly on the Pliocene diatomites. Before gorge incision the deposits east of the present falls were co-extensive with those to the west.

The continuous sedimentation in Lake Gadeb means that any variations in the lake flora must reflect changes in water composition and temperature. The lake was already very low before deposition of the welded tuff, and evolved into a series of shallow swamps thereafter. Fluviatile downcutting to nearly 20 m below the level of the nearly horizontal tuff caprock was followed by deposition of some 10–15 m of Pleistocene alluvium in the centre and west of the Pliocene lake basin. Renewed downcutting is evidenced by two erosional terraces cut into the diatomites near Melka Likimi (Figs 1 and 4), and a final phase of aggradation is evident upstream.

The initial phase of downcutting took place between 2.35 and 1.48 Myr ago. The final phase is younger than the deposition of the unconsolidated gravels with Middle Stone Age flakes at locality 8, and is presumably late Pleistocene[18].

Uplift of the Ethiopian Plateau seems to have accelerated during the Cenozoic, although it was episodic rather than continuous[19]. A recent estimate[20] of 0.1 mm yr^{-1} for the late Pleistocene would imply, if valid also for the southeastern Plateau, an uplift of perhaps several hundred metres since the first hominids occupied the plain of Gadeb.

Tectonic and climatic implications

The above discussion suggests several consequences. First, that major erosion of the Webi gorge may be comparatively recent, although initial downcutting is certainly of late Pliocene to early Pleistocene age. Second, if uplift has indeed accelerated from early to late Pleistocene times, we might expect to find at Gadeb a progressive increase in montane forest pollen with time. Superimposed on this trend would be the pollen fluctuations related to glacially controlled changes in temperature and precipitation. A detailed comparison between pollen spectra and the Pliocene diatom assemblages of Lake Gadeb should allow us to separate tectonically induced changes in lake level from those directly related to regional climatic oscillations. Such analyses are now in progress.

Conclusions

An uninterrupted sequence of diatomites accumulated in a Pliocene lake (Lake Gadeb) which formed when lava flows ponded the drainage of the Webi Shebele in the uplands of south-central Ethiopia some 2.7 Myr ago. The lake shrank progressively until shortly before 2.35 Myr ago, when tuff from the Ethiopian Rift covered the lake deposits and preserved them from erosion. Analysis of the Gadeb diatoms constitutes the first study in Africa of a thick, continuous and well dated lacustrine sequence. As such, it is of great biostratigraphic interest, and will enable correlations to be made with Plio–Pleistocene diatom floras elsewhere in Africa[21–23] and Europe[24–26]. As the lake level fell, rivers from the adjacent uplands deposited sand and gravels across the former lake floor. Hominids occupied the plain between ~1.5 and (?) 0.7 Myr ago, using mostly local sources of basalt and welded tuff to manufacture their implements. Final downcutting by the Webi through the tool-bearing gravels and lake diatomites, and erosion of the Webi gorge, seem to be related to accelerated tectonic uplift during the later part of the Quaternary.

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Hominid occupation of the East-Central Highlands of Ethiopia in the Plio–Pleistocene

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Stone artefacts and fossil evidence suggest that hominids did not reach the high plateau of Ethiopia until ~1.5 Myr ago. Contact between hominids of the plateau and the Rift Valley seems likely.

THE archaeological localities described here, excavated during the winters of 1975 and 1977, are situated in the Plain of Gadeb on the South-East Plateau of Ethiopia at elevations between 2,300 and 2,400 m in the upper reaches of the Webi Shebele River (see Fig. 1 of ref. 1). Except where human interference has disrupted it, the present vegetation pattern of short, montane grassland with stands of *Acacia* and occasional relict junipers and, in the mountains, *Podocarpus* and juniper forests, is not greatly different from that of the Plio–Pleistocene. The long and very complete sedimentary sequence and its palaeoclimatic and palaeoecological interpretation is summarised by Williams *et al.* in the accompanying article[1].

No stone artefacts or other signs of hominid activity have been found in the earlier part of the succession and cultural evidence appears only when the Pliocene Lake Gadeb had mostly dried out, following the breaching and down-cutting of the dam and the formation of the gorge, at a date between ~1.5 and >0.7 Myr on K/Ar and palaeomagnetic reversal evidence (G. H. Curtis and A. Cox, personal communication). The artefacts occur on palaeosols adjacent to the banks and in association with the gravels of shallow stream courses draining into the ponds and swamps that still occupied the lower and central parts of the basin. The cultural horizons are exposed by the meanders of the main river and so occur in the more central parts of the exposed floor of the former Pliocene lake. Examination of the southern flanks near the base of the Bale Mountains (which rise to over 4,000 m) produced only occasional artefacts of Developed Oldowan type suggesting that the more outlying parts of the basin may have been less favourable living places for hominids.

The evidence from Gadeb suggests that hominids appeared on the Ethiopian high plateau only after ~1.5 Myr perhaps ~1.0 Myr ago, at a time when *Homo erectus*, the maker of the Acheulian and Developed Oldowan industrial complexes[2,3], was moving out of the drier and warmer, though well-watered, more low-lying traditional habitats in the Rift Valley and on adjacent parts of the African plateau and was beginning to colonise most ecological niches in Africa—including the high plains.

The Pliocene sediments exposed in the central parts of the basin appear to have been mostly laid down in standing water and so are unlikely to have artefacts associated with them. Only towards the western end, at geological sections 24 and 25[1], is a palaeosol preserved beneath a capping of welded tuff dated to 2.35 Myr (G. H. Curtis personal communication). This palaeosol has yielded part of an elephant molar, perhaps an early form of *Elephas recki*, but the horizon must be examined before we can be certain that no artefacts or other evidence of hominid activity are associated with it.

Developed Oldowan occurrences

The main artefact localities are shown in the accompanying article[1], the most important being localities 2, 8 and 25 and those that line the eastern shoulder of the Webi Gorge downstream from the Falls to the Hako confluence. With the possible exception of the Acheulian artefacts from the gravels above the Gorge, which cannot, as yet, be tied into the stratigraphic sequence upstream, the oldest assemblages recovered occur at locality 2 and come from the iron-stained base of the ferruginous gravels (f) that rest on the mudstones (e) which, in turn, overlie disconformably the Pliocene diatomite. A small excavation, 2A, showed that artefacts and bone fragments occurred in a relatively limited area of 6×2.5 m and, as they are mostly moderately abraded, were not in primary context. The artefacts comprise a handaxe, choppers, core-scrapers, polyhedrons, irregular hard-hammer flakes and the cores from which they were struck, together with a few regularly retouched tools, all from basalt cobbles. A selection is shown in Fig. 1. The assemblage is provisionally classified as Developed Oldowan but the retouched scrapers show a refinement more usual with the Acheulian tradition. Although similar occurrences of choppers,

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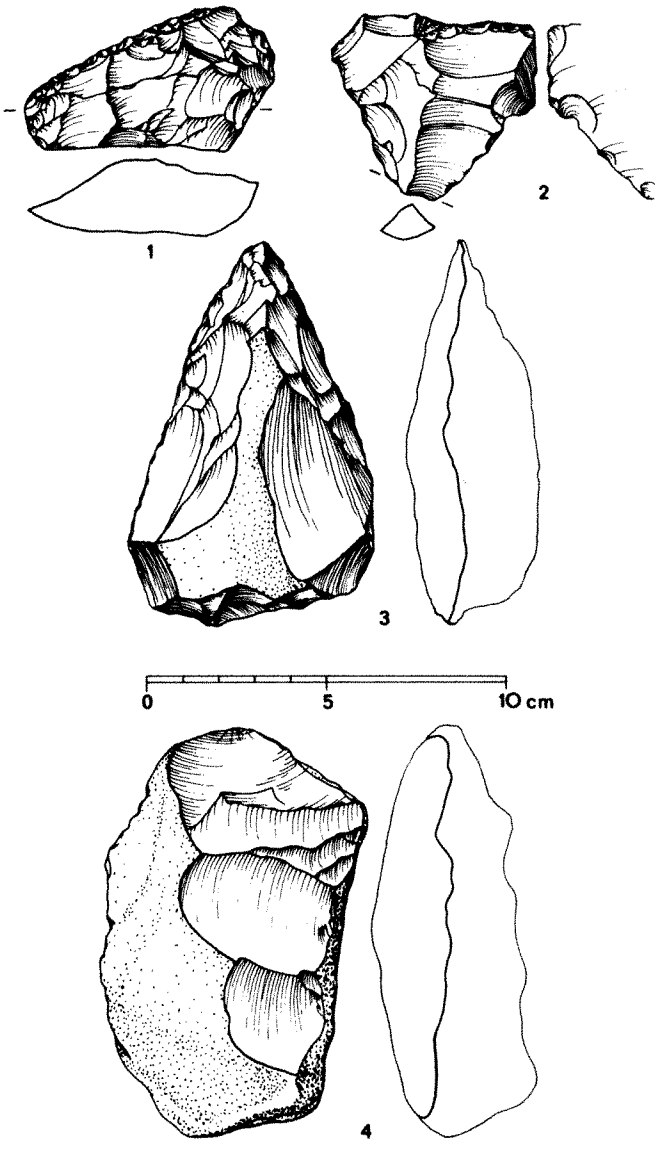


Fig. 1 Artefacts of Developed Oldowan B. tradition from Excavation 2A at Gadeb Locality 2. 1, Side scraper; 2, modified and retouched side-flake; 3, sub-triangular handaxe; 4, side chopper, modified. Material—basalt.

heavy and light duty scrapers and polyhedrons are known from other late Lower and Middle Pleistocene localities in Africa, these are, perhaps, the first such assemblages to have been assigned to the Developed Oldowan B outside the type locality at Olduvai Gorge.

The next oldest Occurrence excavated is found several hundred metres east of 2A, in a channel fill cut into a pumice mudflow dated to 1.48 Myr (G. H. Curtis, personal communication). This channel is 30–40 m wide and filled with alternating deposits of silt, fine sand and clays with occasional thin, discontinuous layers of basalt cobbles, rounded angular fragments of ignimbrite, artefacts and bone in a matrix of sand and fine gravel. An excavation (2E) of 40 m^2 was made in one such horizon towards the eastern edge of the channel and uncovered a hominid activity area, apparently occupied at a time when the channel floor was temporarily exposed. The horizon dips gently from east to west towards the centre of the channel where the gravel is ~10 cm thick but, towards the bank the artefacts and cobbles rest directly on the underlying clayey silt. The silts and clays overlying this horizon contain many pollens and diatoms and the extent to which this Occurrence has been reworked by fluvial action can be assessed by the fact that the diatoms still preserve their fragile spiney structure which would undoubtedly have been broken had there been any turbulence in the water that laid down these beds. Most of the artefacts are slightly (basalt) to moderately (welded tuff) abraded, suggesting that 2E represents a primary context site which has suffered some but no extensive resorting before being fairly rapidly covered by renewed depositional activity.

The assemblage, 1741 specimens, is typical of the Developed Oldowan B Complex (pages 258–281 of ref. 2), comprising the usual range of choppers, core-scrapers, polyhedrons and subspheroids, flake scrapers and modified pieces together with cores, flakes and flaking waste (Fig. 2). There are also six handaxes and one cleaver, all exhibiting hard-hammer technique and showing a lack of the refinement normally found with the Acheulian assemblages at Gadeb. Note that unmodified cobbles comprise only 18.4% and simply split cobbles 4.2% of the total. If the assemblage were all fluvially deposited one would have expected many more cobbles than artefacts which suggests that the former may have been carried onto the site by the hominids to use for making tools.

A number of fossil bone fragments and teeth, ranging from fresh to moderately abraded, are associated with the artefacts. Bones, mostly broken into small pieces, and teeth came from hippopotamus, elephant, various bovids, zebra and pig. No concentrations could be discovered but the assemblage is an indication of the game animals available on the high plateau at this time (faunal identifications at Gadeb by D. Geraards and S. Edwards are provisional).

Higher in the 22-m sequence at Locality 2, are stratified basalt gravels with artefacts, overlying weathered tuffs. Two small excavations made in 1975 exposed parts of two further concentrations of Developed Oldowan B character, both in channel fills with, in each case, no significant resorting by subsequent stream action. The earlier occurrence (2C) rested on the weathered tuffs and was covered by fine, current-bedded sands ~1 m thick. A total of 622 artefacts, with some small, unidentifiable bone fragments, came from a 3.5 m × 1.5 m excavation and comprise 12.5% shaped and modified pieces and 87.4% unmodified waste: 66 are shaped tools—choppers, polyhedrons, 5 rather poorly-made handaxes, 4 other bifacially worked artefacts and 27 small scrapers on flakes, flake fragments and chunks, the commonest form being a single-side scraper. There are also 12 pieces with modified edge damage. The artefacts showed no discernable orientation or concentration and were fresh to moderately abraded.

The 2B site, a little higher in the basalt gravels, yielded 589 artefacts from a 2 × 4 m excavation, shaped tools representing 9.3%, modified 0.3% and unmodified waste 90.3%. Again the assemblage can be classified as Developed Oldowan B with the usual choppers, polyhedrons and scrapers. There are also one proto-biface, one handaxe and one cleaver, again poorly-made in comparison with those from the Acheulian. When compared, all three of these Locality 2 assemblages (2B, 2C and 2E) are seen to be similar. In addition, comparison with assemblages of this Complex from East Africa confirms that the Gadeb occurrences belong with the Developed Oldowan B, or perhaps C (from Beds III and IV at Olduvai Gorge), although this has, as yet, not been described in detail[4–6].

Acheulian occurrences

The earliest Acheulian assemblages yet found at Gadeb occur at Locality 8 associated with basalt gravels and felspathic sands resting on an erosional unconformity cut down into the later Pliocene diatomites. This horizon has been excavated in three places—two (8A and 8D) in 1975 and the third (8E) in 1977[1]. The first two were moderately small but that at 8E exposed ~100 m^2 of the occupation horizon (Fig. 3). Again, the context is a shallow stream channel cut across the floor of the Pliocene Lake Gadeb and draining into the central lakelets and swamp. The small 8A excavation produced the surprising number of 1,849 artefacts of which 251 were handaxes and cleavers, most

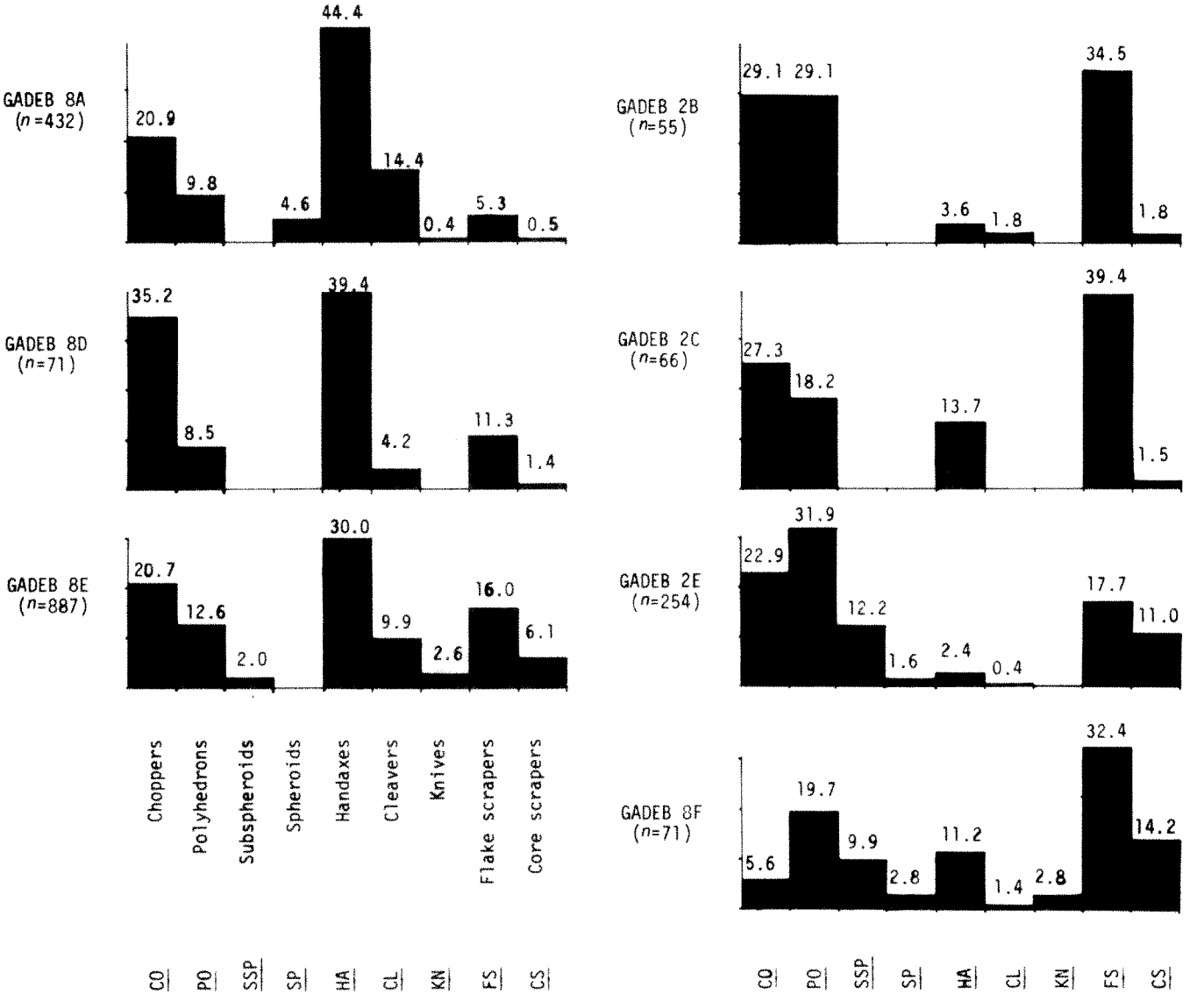


Fig. 2 Histograms comparing Acheulian (left) and Developed Oldowan (right) tool-kits from excavated sites at Gadeb, east central Ethiopia. Each division on the vertical axis represents 10 per cent.

moderately to heavily abraded and their alignment and imbrication show clearly that the assemblage had been incorporated in the stream gravels. Excavations 8D and 8E were ~30 m apart and exposed the edges of a channel and its banks to the south-east. To the south and west the gravel was 10–15 cm thick while at the eastern end the artefacts rested directly on the diatomite. The 8D excavation (3×5 m) revealed a primary context concentration of artefacts and fossil bone, including two complete hippopotamus canines and fragmentary remains of bovids, zebra and elephant as well as unidentifiable bone fragments. The 487 artefacts were mostly fresh to slightly abraded and 14.6% were shaped tools, 1.6% modified and 83.8% unmodified waste. Bifaces comprise 43.6% of the shaped tools—mostly handaxes with three cleavers—the remainder being flake scrapers, choppers and spheroids indistinguishable from similar tools associated with the Developed Oldowan.

The 8E excavation in the same occupation surface yielded 20,276 artefacts and a small quantity of fauna. Except for a nearly complete zebra mandible, the bone consisted mostly of unidentifiable small fragments and isolated teeth, the commonest of these being hippo represented by two complete, large incisors and several molars and molar fragments. The next commonest group were the bovids followed by zebra. Again, there were no bone accumulations and it would appear that butchery was not a major activity here or at the other Acheulian sites excavated at Gadeb.

The greater number of artefacts occurred in the northern half of the excavation due, probably, to gravitation down-slope. No preferred orientation is apparent and the bifaces lay flat on the surface (Fig. 3). 45% of artefacts are moderately, and 26% slightly abraded; 15% are fresh. It seems that the assemblage has suffered slight movement, though insufficient to disperse or significantly alter the concentration before it was buried under felspathic sands with silt and clay lenses in the lower part.

The composition of the 8E assemblage is given in Fig. 2: 4.4% of the pieces are shaped tools, 10.5% are modified or show edge damage and 50% is unmodified waste the remaining 35% being cobbles and split cobbles. The commonest shaped tools are bifaces: handaxes (25%), cleavers (9.9%), knives (2.6%) and other (or broken) bifaces (4.9%). The next most numerous are flake scrapers (16.0%) among them several continuously retouched and convergent forms. The other tools—choppers, polyhedrals, spheroids and core scrapers—are similar to those found with the Developed Oldowan (Fig. 4).

The raw material is basalt and ignimbrite with a little chert and obsidian. The ignimbrite was obtained from outcrops, the nearest today being several kilometres to the west and south. The ignimbrite handaxes were made from large flakes struck

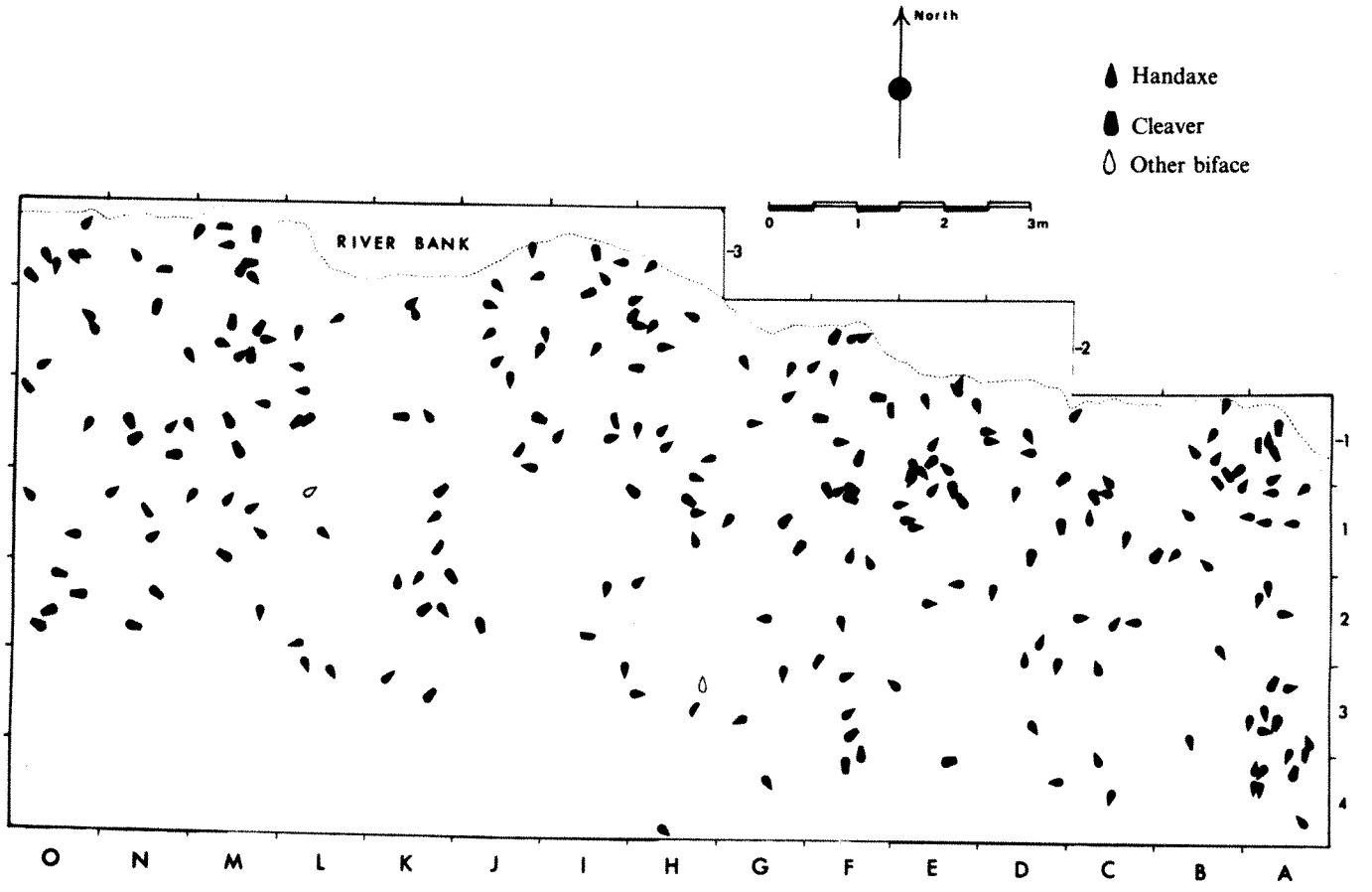


Fig. 3 Plot of the distribution and orientation of the bifaces (handaxes, cleavers) on the Acheulian occupation horizon at Gadeb 8E, 1977.

from these outcrops and are larger, broader, thicker and more elongated than those made from basalt cobbles which are generally ovate or long ovate in plan form (Fig. 4, no. 9). The large flakes from cobbles, retouched as handaxes and cleavers, usually have a flat release face which often results from splitting a cobble by throwing (N. Toth, personal communication). Bifacial handaxes are commonest but an interesting form occurs flaked only on the release face and exhibiting inverse retouch; occasionally, these are also minimally retouched on the cortex face. Flake scrapers also sometimes show this form of retouch. (Biface plan forms in relation to raw material at 8E are shown at Fig. 5).

Four ovate handaxes in obsidian from 8E are of special interest since they—or the raw material—must have come from a considerable distance. No sources of obsidian are known on the South-east Plateau the nearest being to the west in the Rift or on the escarpment edge ~100 km away[1]. This distance is appreciably greater than any hitherto known for the Lower Palaeolithic and has important implications, therefore, for movement or group contacts between the Plateau and the Rift.

Among the modified or utilised artefacts are 11 cobbles, or rounded fragments, with a pit formed in the centre of one face by hammering with some pointed implement (Fig. 4). Similar pitted stones have been found at Olduvai Gorge (upper Bed II and Beds III/IV)[7], and on the Developed Oldowan horizon Gomboré I at Melka Kunture on the Ethiopian Plateau[8]. They are believed to be anvils used with hammer stones in the bipolar flaking method although other uses cannot be excluded.

The occupation floor also yielded several fragments of heavily weathered basalt which, when rubbed, give a red pigment. None of the pieces show unquestionable evidence of rubbing but the possibility should not be ignored. Several pieces of welded tuff showing what appears to be alteration by fire were also recovered, including several together in a concentration. Palaeomagnetic measurements by Dr M. Barbetti, University of Adelaide, on samples from the 8E activity area have produced suggestive, but not conclusive evidence for the use of fire by Acheulian man at Gadeb.

Butchery site

On the surface of the palaeosol overlying the felspathic sands covering the Acheulian occupation at Locality 8 (Fig. 3, ref. 1), was found, at Excavation 8F, a discrete concentration of artefacts associated with part of a butchered hippo (Fig. 6). The 385 fresh or slightly abraded artefacts are presumed to be in primary context; 18.4% are shaped tools, 7.8% are modified or show edge damage and 3% are miscellaneous pieces (Fig. 2). Only 51.2% represent unmodified waste and 19.2% are cobbles or split cobbles all of which must have been carried onto the site. The commonest tools (32.4%) are small flake side-scrapers; polyhedrons and spheroids also account for 32.4% and there are 14.1% of core-scrapers. Choppers are few (5.7%) and bifaces are represented by a cleaver, three small, poorly-made handaxes, two knives, similarly of crude workmanship and five miscellaneous forms. Among the modified/utilised artefacts are 17 flakes and flake fragments. This tool-kit could have been used in butchering the hippo and the polyhedrons, spheroids and some of the small tools were found in close association with the bones.

Technically this assemblage belongs and is assigned to the Developed Oldowan B complex. The palaeosol is overlain by silts with reversed magnetism and has an age of $\geq$0.7 Myr.

Quarry site

An important Acheulian ignimbrite quarry and workshop was discovered at the end of the 1977 season at Locality 25, ~6.5 km west of locality 2 where basalt gravels, overlain by ash and

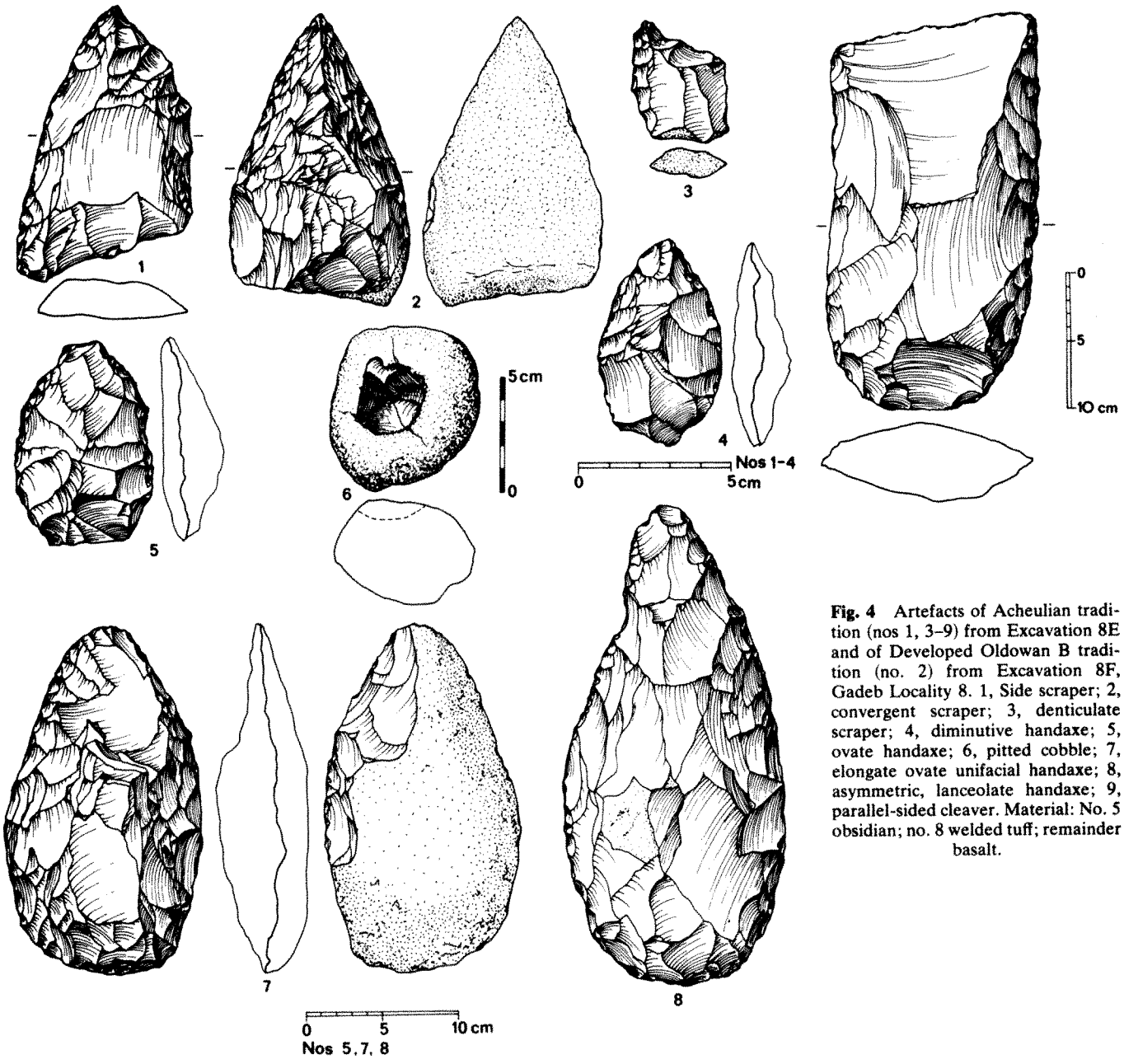


Fig. 4 Artefacts of Acheulian tradition (nos 1, 3–9) from Excavation 8E and of Developed Oldowan B tradition (no. 2) from Excavation 8F, Gadeb Locality 8. 1, Side scraper; 2, convergent scraper; 3, denticulate scraper; 4, diminutive handaxe; 5, ovate handaxe; 6, pitted cobble; 7, elongate ovate unifacial handaxe; 8, asymmetric, lanceolate handaxe; 9, parallel-sided cleaver. Material: No. 5 obsidian; no. 8 welded tuff; remainder basalt.

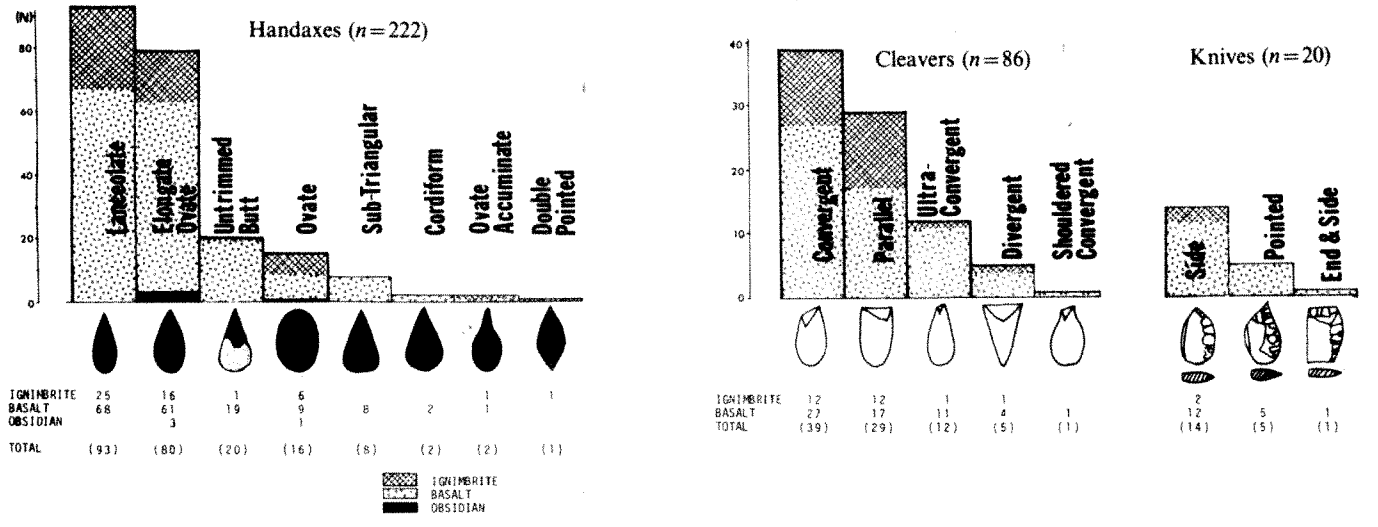


Fig. 5 Acheulian biface forms and raw materials at Gadeb 8E, 1977.

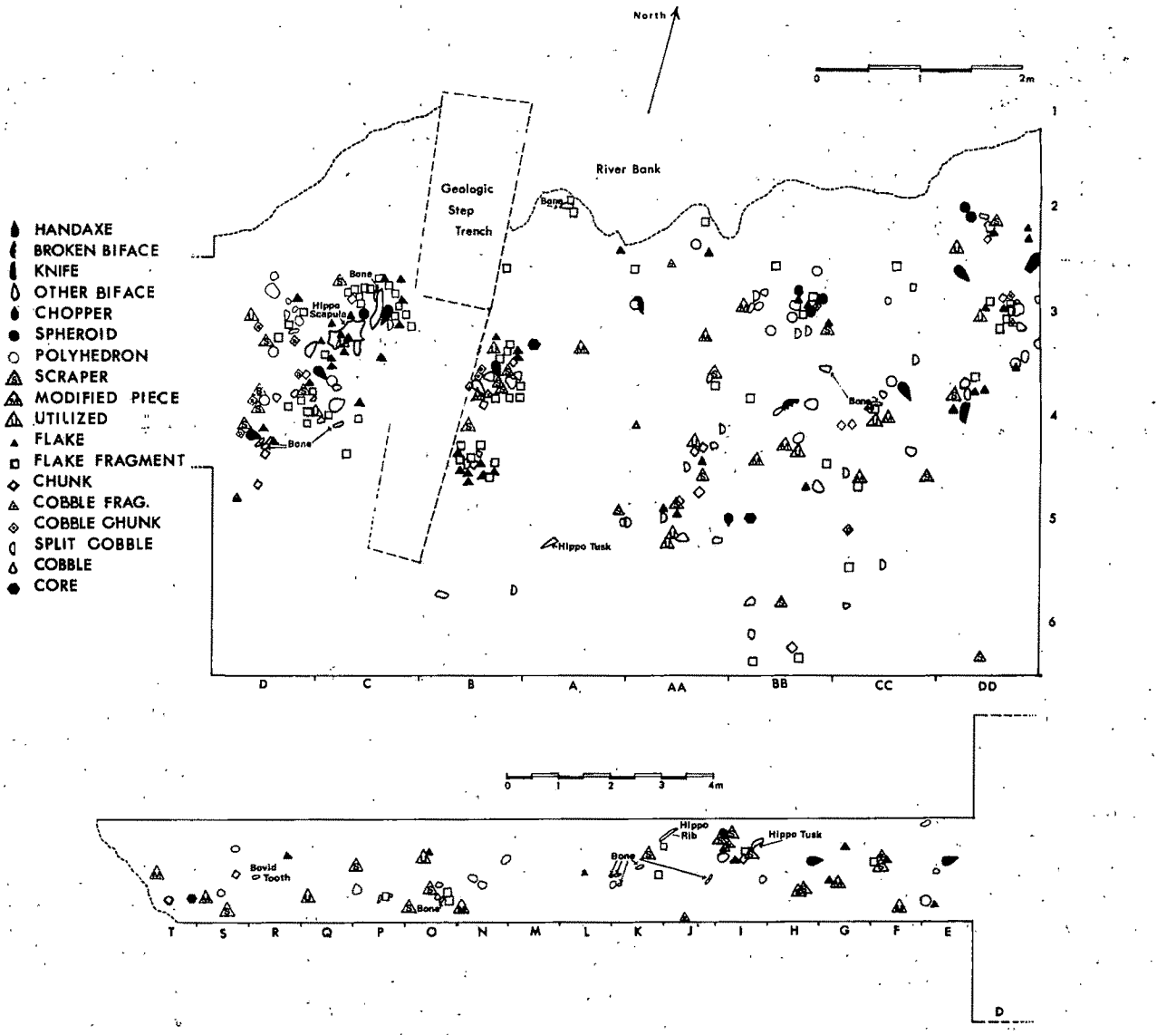


Fig. 6 Plan of the hippo butchery concentration on the palaeosol at Gadeb 8F, 1977.

pumice sandstone, fill an erosion channel cut into the earlier Pliocene sediments capped by welded tuff (Fig. 5 of ref. 1). A small trial trench yielded 112 Acheulian artefacts in fresh condition together with fossil bone. The ignimbrite had been 'mined' by knocking off large flakes from the outcrop and fallen blocks and there is no evidence of the proto-Levallois technique. This is one of the few sealed Acheulian quarry sites known to us but, without further work, it cannot be correlated directly with the succession at localities 2 and 8.

Either contemporary with or somewhat later than the 8F butchery site are two well-made, fresh Acheulian cleavers with some fossil bone which had come from the base of the bedded pumice sands (j) in the deep channel fill beneath the 'upper laminites' (m) at locality 2 (Fig. 3 of ref. 1). These are the youngest of the Lower Pleistocene Occurrences at Gadeb, as no tools have, as yet, been found in the Pumice Sandstone that caps the succession.

In the gravels at the top of the Webi Gorge between the Falls and the Hako confluence, concentrated patches of Acheulian bifaces and other tools occur in secondary context. At the Hako confluence itself, residual gravel patches yielded weathered, but not especially water abraded, choppers, cores and flakes of Developed Oldowan form. Clearly, therefore, Acheulian man was present during the final breaching of the dam and while the river was cutting back upstream as far as the lower of the two existing waterfalls.

Conclusions

It seems probable that hominids did not penetrate to the high plateau until after the Acheulian complex appeared in the Rift after 1.5 Myr ago. The Gadeb sites show an alternating sequence of Acheulian and Developed Oldowan occurrences dating between ~1.5 and >0.7 Myr. These must have been contemporary traditions the main distinguishing feature being the bifaces with the Acheulian, whose makers were, therefore, engaged in biface-related activities not common with the Developed Oldowan. The bone waste found on the sites, however, indicates that butchery was more commonly practised by the makers of the Developed Oldowan using the chopper, polyhedron and small tool element. The sites were generally close to or in stream courses, as elsewhere in East Africa and Ethiopia[9,10] and, while remains of grazing animals are found, the preferred food animal appears to have been the hippo. The Gadeb assemblages show generally close similarities, though with local variation, with those from sub-Saharan Africa as a whole.

The hominids seem to have been relatively far ranging and contact between plateau and Rift seems likely. *Homo erectus*, even at this unusually early time, may have been experimenting with pigment at Gadeb and also with fire; the latter, perhaps, induced by the cold temperature of the plateau since this must be one of the highest altitudes at which assemblages of this time range have been found in either Africa or Eurasia.

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Isolation and characterisation of a yeast chromosomal replicator

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A yeast DNA sequence that behaves as a chromosomal replicator, ars1 *(autonomously replicating sequence), has been isolated. On transformation,* ars1 *allows autonomous replication of all co-linear DNA. The replicator can integrate into other replication units and can function in multimeric form. The 850-base pair* ars1 *element has no detectable homology to other yeast sequences. Such replicator-containing plasmids can be used for the isolation of DNA sequences in yeast cells as well as for the study of chromosomal DNA replication.*

THE replication of a cell's genome must be properly controlled to harmonise with the many other functions required for cell division. In bacteria, the rate of DNA replication is controlled at the onset of synthesis[1,2]. The initiation event occurs at a single site on the bacterial chromosome[3]. This initiation site or origin of replication has been genetically defined as the 'replicator' (ref. 4), an element that allows replication of all DNA attached to it. Several prokaryotic replicators have been isolated (see ref. 5 for examples) and their further biochemical and genetic analysis will help elucidate the molecular mechanism(s) that control the initiation of bacterial DNA synthesis.

How is DNA replication controlled in the larger genomes of eukaryotes? An abundance of physical data indicate that eukaryotic chromosomes are organised into tandem replication units (reviewed in refs 6–8). The rate of DNA chain elongation seems to be the same for all the replication units in a cell, whereas different units initiate DNA synthesis at different times. Furthermore, initiation is a non-random event. Replication units begin synthesis in a defined order and individual units demonstrate the same temporal specificity in successive divisions. However, the paucity of genetic and biochemical evidence means that it is impossible to determine whether such replication units are strictly analogous to prokaryotic chromosomes. For example, it has been proposed that initiation occurs not at a specific DNA sequence, but at a random site, determined solely by the spatial arrangement of the DNA within the highly structured chromosome[6,7].

During our studies on transformation of the yeast, *Saccharomyces cerevisiae*, we identified a segment of chromosomal DNA that could replicate without integrating into the yeast genome[9]. Here we further characterise this autonomously replicating segment. Its behaviour suggests that the DNA sequence contains a eukaryotic chromosomal replicator.

*ars*1: A chromosomal replicator

A 1.4-kilobase pair *Eco*RI endonuclease-generated DNA fragment, containing the centromere-linked *trp*1 gene was isolated (D.T.S. and R. W. Davis, in preparation). This fragment (Sc4101) was inserted into the *Eco*RI cleavage site of the bacterial plasmid, pBR322 (ref. 29) (Fig. 1). The resulting plasmid is termed YRp7. Covalently closed, supercoiled YRp7 DNA was used to transform a *trp*1$^-$ yeast strain. The efficiency with which these cells were transformed to Trp$^+$ was approximately 1,000-fold higher than the efficiency observed with other segments of yeast chromosomal DNA[9]. Furthermore, the high frequency of transformation was associated with autonomous replication. When DNA was isolated from these transformants and analysed by restriction endonuclease digestion and DNA–DNA hybridisation, the transforming sequences were detected as supercoiled, circular DNA and were not found integrated into the host genome. Presumably, the higher frequencies of transformation arise because autonomous replication allows heritable expression without requiring chromosomal integration via a less frequent recombination event. Thus, high transformation efficiency can be used as an indicator for autonomous replication of these hybrid molecules.

To test whether autonomous replication might be due to the particular sequences at the yeast/pBR322 junctions, the transformation efficiency of YRp7′, which contains the Sc4101 insert inverted relative to YRp7, was measured. Both YRp7 and YRp7′ gave rise to 2,000–4,000 Trp$^+$ colonies per μg DNA. As other yeast sequences (for example the *his*3, *leu*2, *ura*3 structural genes[9,11,12]) fail to replicate autonomously when inserted into pBR322, it is likely that the element that permits autonomous replication is located on Sc4101 itself. This was further investigated by inserting the Sc4101 DNA fragment into YIp1, a *his*3-containing yeast vector that transforms at low efficiency via integration into the host chromosome (Fig. 1). As expected, the frequency at which YRp7–Sc2605 and YRp7′–Sc2605 DNAs transformed *his*3$^-$ to His$^+$ was also high (2,000 His$^+$ colonies per μg DNA). Sc4101 has been joined to many

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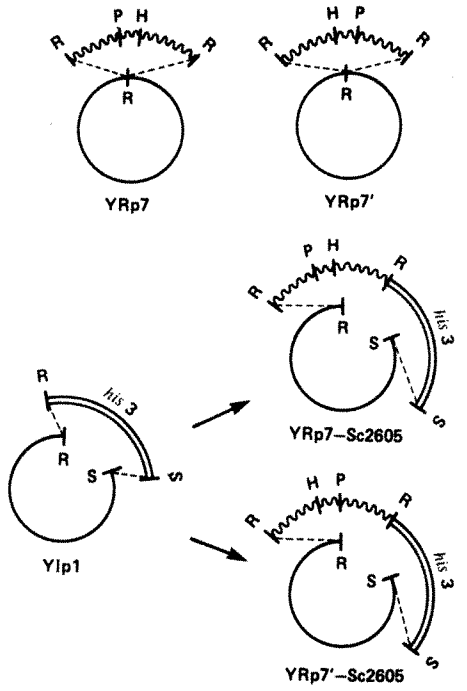


Fig. 1 Structure of several yeast replicator plasmids. Solid lines depict pBR322 plasmid sequences, wavy lines represent the *trp*1-containing 1.4-kilobase pair yeast fragment (Sc4101) and open bars a 6.0-kilobase pair yeast fragment (Sc2605) that contains the *his*3 structural gene. Not to scale. YRp7 and YRp7′ were constructed by the ligation of an *Eco*RI digest of λgt-Sc4104 and pBR322 as described previously[9]. YRp7–Sc2605 and YRp7′–Scα605 were similarly constructed by insertion of the Sc4101 fragment in either orientation into the *Eco*RI cleavage site of YIp1. *Eco*RI was the gift of Marjorie Thomas. Other restriction endonucleases and T4 DNA ligase were from New England Biolabs or Bethesda Research Laboratories and were used as directed. After ligation, the hybrid molecules were transformed[31] into trpC9830, an *E. coli* strain lacking *N*-(5′-phosphoribosyl)anthranilate isomerase. The presence of Sc4101 was then determined by testing for complementation of the bacterial lesion by the yeast *trp*1 gene (D.T.S. and R. W. Davis in preparation). The orientation and fidelity of the purified plasmid DNA were assessed by restriction endonuclease digestion and horizontal agarose gel electrophoresis[10]. Transformation of D13-1A (*a trp*1 *his*3-532 *gal*2 *SUC*2 *mal*$^-$) was carried out as described previously[9]. The transformation efficiencies for the YR plasmids cited in the text were determined on several separate occasions using other yeast vectors to control for experimental variation.

other yeast and bacterial sequences, producing hybrid molecules 4 to 50 kilobase pairs in total length. All these Sc4101-containing DNAs exhibited high frequency transformation and, concomitantly, replication in the absence of recombination with the host genome (S. Scherer, T. P. St. John, D.T.S. and K.S., unpublished observations). These data are consistent with the idea that Sc4101 contains, in addition to the structural sequence for the *trp*1 gene, a genetic element that allows autonomous replication, which we will call *ars*1 (for autonomously replicating sequence).

Yeast cells can be transformed simultaneously by two different DNA species at high frequency[13]. For example, when a mixture of YRp7–Sc2605 and YRp12 (YRp7′ with a 1 kilobase pair yeast DNA fragment carrying the *ura*3 gene inserted at the *Ava*I endonuclease cleavage site[12]) DNA was added to a *ura*3$^-$*his*3$^-$ strain, Ura$^+$His$^+$ co-transformants arose at the same frequency (2,000–5,000 colonies per μg) as Ura$^+$ or His$^+$ transformants. So, in these transformation conditions, DNA is saturating and each yeast cell is capable of receiving multiple DNA molecules. This observation can be used to design a *cis*/*trans* test for *ars*1 function. If *ars*1 functions in *trans*, co-transformation of a *his*3$^-$*trp*1$^-$ yeast strain by YIp1 and YRp7 DNAs should lead to a 1,000-fold increase in His$^+$ colonies compared with transformation by YIp1 alone. However, the mixture of YIp1 and YRp7 DNA gave rise to only 78 His$^+$ colonies per μg DNA. Concurrently, YRp7–Sc2605 DNA gave rise to 2,000 His$^+$ colonies per μg DNA. Thus Sc4101 allows high frequency transformation and autonomous replication of the *his*3 sequence only when located on the same molecule. As *ars*1 is linked to the yeast chromosomal *trp*1 locus and behaves as a *cis*-acting effector of DNA replication, we propose that *ars*1 is a yeast chromosomal replicator.

Autonomously replicating *ars*1 hybrid molecules are unstable

In a minimal medium supplemented with histidine and tryptophan, a *trp*1$^-$ *his*3$^-$ yeast strain will grow with a doubling time of 2.5 h. When transformed by YRp7–Sc2605 to His$^+$Trp$^+$, the strain doubles in 3.5–4 h in the absence of histidine and/or tryptophan. Similarly increased generation times are observed for all other *ars*1-containing transformants, regardless of the selective marker used. It is thus unlikely that the slow growth rate is due to a deficiency in expression of the transformed genes but is due rather to mitotic loss of the autonomously replicating hybrid DNA. In a logarithmically growing minimal medium culture of a *his*3$^-$ strain transformed by YRp7–Sc2605 DNA only 30–50% of the cells are phenotypically His$^+$. If this culture is diluted into a non-selective medium, the proportion of His$^+$ cells drops to 1–5% within 10 generations. The resulting *his*3$^-$ cells do not contain any hybrid DNA as determined by DNA–DNA hybridisation (data not shown).

There are several possible explanations for the mitotic instability of *ars*1-containing hybrid molecules. The isolated replicator may not contain all the information necessary for complete replication during each cell cycle. However, larger DNA fragments that contain additional DNA sequences adjacent to *ars*1 are still mitotically unstable (D.T.S. and R.W.D., in preparation). Alternatively, *ars*1-containing extrachromosomal elements may compete with the host chromosome for limited initiator(s). This is one proposed explanation for the instability of plasmids containing the *Escherichia coli* origin of replication[15,16]. Hybrids containing *ars*1 plasmids may replicate during each cell cycle but may fail to segregate properly during mitosis. In the last case, addition of other DNA sequences controlling chromosomal segregation would be expected to stabilise a replicator plasmid.

Intregation of *ars*1 hybrid molecules

The slow growth of strains containing *ars*1 hybrid molecules results in a strong selective pressure favouring any stably transformed cells. Such transformants can indeed be isolated. After many generations of growth in selective or non-selective conditions, cells were plated on non-selective medium. These colonies were tested for the stability of the transformed phenotype by replica plating to a selective medium. Stable transformants isolated in this way grew at the parental rate in minimal medium. The fate of the transforming DNA was investigated in four independently isolated stable strains (Fig. 2). The diagram at the bottom of Fig. 2 shows the digestion patterns expected for three possible conformations of the foreign sequences. The transforming DNA was identified by hybridisation and co-electrophoresed with the high molecular weight linear host DNA in all four cases (Fig. 2, lanes 1–4). The structure of the integrated DNA in one transformant was analysed by digestion with *Bam*HI endonuclease. The foreign pBR322 sequences in the stable transformant were detected as a single band (Fig. 2, lane 7) co-migrating with the *Bam*HI fragment from an unstable transformant (lane 6) and from the original plasmid DNA (lane 9). These data indicate that YRp7–Sc2605 is integrated at the *his*3 locus without major sequence alteration.

Given the appropriate genetic markers, stable transformants may be directly selected. Fragments containing non-complementing derivatives of the *his*3 gene (the missense mutations *his*3-38 and *his*3–532 (ref. 17), as well as an amber mutation[18] and two deletions[19]) were inserted into YRp7. These hybrid molecules were introduced into a *his*3-532*trp*1$^-$ strain by transformation to Trp$^+$. Although the transformants were initially His$^-$, His$^+$ recombinants could be selected at a frequency of 10^{-5}–10^{-6} per generation. The only exception to this result

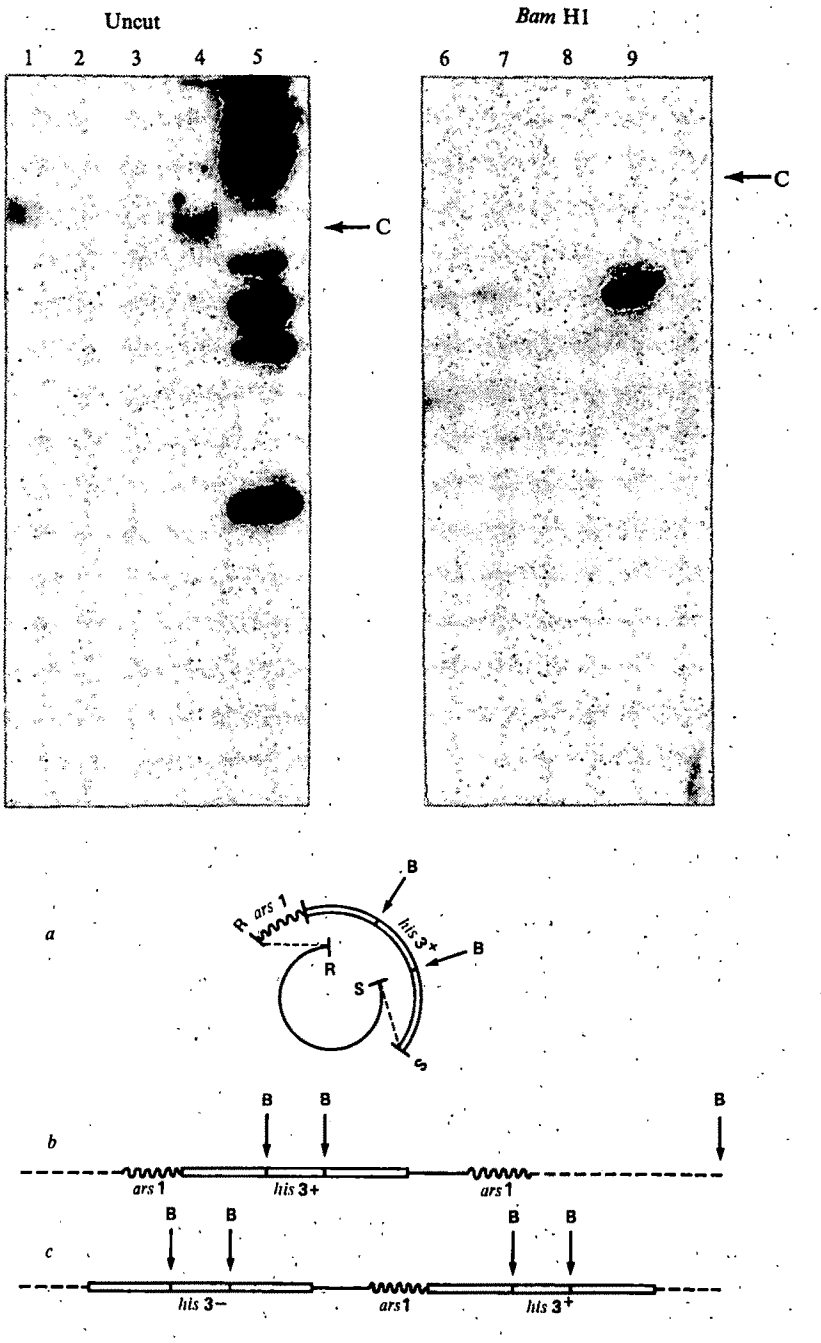


Fig. 2 Analysis of stable *ars*1 transformants. DNA was rapidly purified from transformants grown in minimal media (6.7 g l^{-1} Difco yeast nitrogen base, 2% glucose) as described previously[9]. In 'uncut', the DNA from four stable transformants described in the text was electrophoresed without endonuclease digestion in a 0.4% agarose gel (lanes 1–4). Lane 5 contains the original transforming DNA. The multiplicity of bands (several of which showed lower mobilities than the yeast chromosomal DNA) is apparently due to multimeric and nicked forms of the plasmid. In '*Bam*HI', DNA isolated from an unstable transformant (lane 6), a stable, integrated transformant (lane 7), the parental strain (lane 8), and the transforming plasmid DNA (lane 9) were digested with *Bam*HI restriction endonuclease and electrophoresed on a 0.4% agarose gel. The DNA in each gel was transferred to a sheet of nitrocellulose paper[25]. ^{32}P-labelled pBR322 DNA was prepared by nick-translation[26], heat denatured, and hybridised to the immobilised DNA spectra. These operations were carried out using modifications of the traditional procedures as described in ref. 27. Pictures of the two autoradiographs are presented here. Also depicted are three possible structures of transformed *ars*1 sequences: *a*, unintegrated and autonomously replicating; *b*, integrated by homologous recombination between the *ars*1 sequence and its chromosomal counterpart; and *c*, integrated by homologous recombination between *his*3 sequences. Letter and line designations are as in Fig. 1 with one addendum: B represents the predicted *Bam*HI endonuclease cleavage sites.

occurred when the transforming DNA contained the same *his*3 allele as the recipient yeast strain (*his*3–532), in which case no His^+ recombinants could be isolated. All the recombinants obtained were stable for both His^+ and Trp^+ and grew at the wild-type rate in minimal medium. Hybridisation analysis (as described above) indicated that the transforming DNA had integrated at the *his*3 locus without detectable sequence alteration (data not shown). The frequency of recombination between the *his*3–38 allele (on the transforming plasmid) and the *his*3–532 allele (in the recipient) was roughly the same as the frequency observed when the two alleles were present in a heterozygous diploid yeast strain. In all respects, the integration of *ars*1-containing hybrid molecules seems to be a simple consequence of mitotic recombination.

The organisation of DNA replication in *Saccharomyces cerevisiae* is similar to that of other eukaryotes. Fibre autoradiography and electron microscopy studies indicate that each chromosome is divided into several replication units and the initiation of DNA synthesis for each unit is rigidly controlled[20–23]. The ability of *ars*1 sequences to integrate has implications for this control. We have shown the DNA sequences containing a replicator can stably integrate at other loci. As *ars*1 is located near the centromere of chromosome IV and *his*3 maps on the left arm of chromosome XV, the two loci are probably in different replication units. The observation that *ars*1 can integrate at *his*3 indicates that either initiation of DNA replication at *ars*1 is suppressed in its new position or translocation of a second replicator simply creates a new functional replication unit.

Multimeric forms of *ars*1

Plasmids containing *ars*1 have also been integrated in the *trp*1–*ars*1 region near the centromere of chromosome IV (data not shown). As such a recombination event creates a tandem duplication, it suggests that multimers of a chromosomal replicator function normally. To investigate this further, we examined the *ars* phenotype of monomer, dimer and trimer YRp7.

When YRp7 is propagated in a recombination-proficient bacterial host, monomer, dimer and trimer forms of the plasmid arise. Each of the three forms of the plasmid DNA was purified

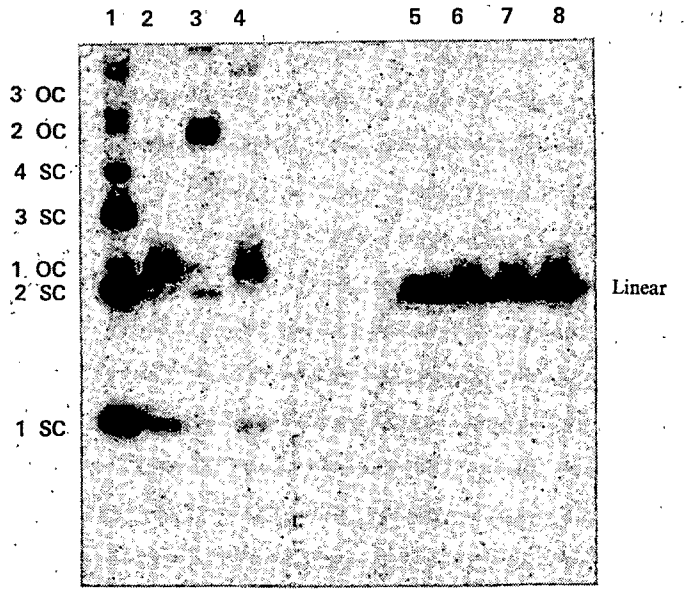


Fig. 3 Autonomous replication of monomer, dimer and trimer YRp7. Plasmid DNA purified from trpC9830 (Rec$^+$) carrying YRp7 was used to transform KM601, a *recA*$^-$*recB*$^-$ *E. coli* strain (gift of Kevin McEntee). Plasmid DNA was rapidly isolated from individual tetracycline-resistant transformants and its size was assessed by agarose gel electrophoresis. Transformants were then picked that contained predominantly monomer, dimer and trimer YRp7. The plasmid DNAs were purified and analysed by restriction endonuclease digestion and agarose gel electrophoresis. Each sample contained some larger multimers, but neither the dimer nor the trimer DNA preparations contained detectable amounts of monomer plasmid and all were free of any gross alterations of the *ars*1 sequences (data not shown). Monomer, dimer or trimer YRp7 DNA was used to transform D13-1A to Trp$^+$. DNA from the transformants was purified, electrophoresed in a 0.4% agarose gel, transferred to nitrocellulose and hybridised to ^{32}P-labelled pBR322 DNA as described in Fig. 2 legend. DNA in lanes 1–4 was uncut while the DNA samples in wells 5–8 were digested with *Bam*HI restriction enconuclease. Lanes 1 and 5 contained 1 μg of wild-type yeast DNA and 2 ng each of purified monomer, dimer and trimer YRp7 DNA. The various multimeric forms are designated by the numbers 1, 2, 3 and 4 (for monomer, dimer, trimer and tetramer) while SC and OC distinguish supercoiled from nicked, open circular DNA. Lanes 2 and 6 each contained 1 μg of DNA purified from D13-1A transformed to Trp$^+$ by the YRp7 monomer form. DNA samples purified from D13-1A transformed by dimer and trimer YRp7 were electrophoresed in lanes 3, 7 and 4, 8 respectively.

by clonal isolation in a *recA*$^-$*recB*$^-$ *E. coli* strain. Transformation of a recombination-proficient *trp*1$^-$ yeast strain to Trp$^+$ occurred at equal frequencies for monomer, dimer or trimer DNA (2,000–5,000 colonies per μg DNA). Strains transformed by each of the three DNAs had identical growth rates and were equally unstable mitotically. The structure of the transforming DNA in such strains was analysed (Fig. 3). Strains transformed with monomer and dimer YRp7 contained, respectively, monomer and dimer autonomously replicating supercoiled circles (lanes 2 and 3). Small amounts of other plasmid forms were detected (dimer in the monomer transformant as well as both monomer and tetramer in the strain carrying the dimer form). A strain transformed with trimer YRp7 DNA contained predominantly monomer plasmid (lane 4). It is unlikely that this isolate arose through transformation by contaminating monomer plasmid in the trimer DNA preparation (see Fig. 4 legend), so appearance of the monomer plasmid probably represents recombinational reduction of the multimeric form. The various circular forms in all three strains contained intact YRp7 sequences as judged by *Bam*HI endonuclease digestion which reduces all multimeric species to a single linear fragment (Fig. 2, lanes 5–8). Thus monomer and dimer YRp7 seem to be functionally equivalent. Some interconversion of multimeric forms occurs, probably by mitotic recombination in the yeast host cells.

A unique feature of *ars*1 is that it is tightly linked to the centromere of chromosome IV. In fact, we considered the possibility that the *ars* phenotype could be caused by a centromeric function. As described above, Sc4101 is capable of both stably integrating at other chromosomal loci and functioning in a dimeric state. If Sc4101 contained a centromere, both of these constructions would represent dicentric molecules which might be expected to undergo sequence alterations when propagated mitotically[30]. As we detected no such alterations, we conclude that Sc4101 is unlikely to have full centromeric function. However, these experiments suggest tests for segregational activity which may lead to the identification of other yeast fragments that contain centromeric sequences.

Delimitation of *ars*1

Sc4101 contains two functions: *trp*1 and *ars*1. To determine the position of the *ars*1 sequences we have constructed several deletions of the 1.4-kilobase pair *Eco*RI-generated fragment. The experimental approach is shown in Fig. 4. Unique restriction endonuclease sites in Sc4101 were fused to unique sites in the pBR322 sequence, deleting parts of both Sc4101 and pBR322. Utilising both YRp7 and YRp7′, it was possible to construct two pairs of complementary deletions; Yp411, Yp413 and Yp412, Yp414. Fragments containing the *ura*3 or *his*3 sequences (known to function when attached to an intact *ars*1 element) were inserted into each of the four deletions. Only Yp413/HIS3$^+$ and Yp412/URA3$^+$ DNAs were capable of transforming *his*3$^-$ and *ura*3$^-$ yeast strains, respectively. Both transformed at high efficiency (again ~2,000 colonies per μg DNA). Yp412/URA3$^+$ had a completely normal *ars*1 phenotype. Strains transformed with Yp413/HIS3$^+$, however, had a greatly reduced growth rate, with a generation time of about 11 h, and an enhanced mitotic instability. Assuming that juxtaposition of the *ura*3 or *his*3 sequences does not effect *ars*1 function, we conclude that the *ars*1 sequence is fully contained within the 850 base pairs between the *Eco*RI and *Hin*dIII cleavage sites and deletion of the 200 base pairs between the *Hin*dIII and *Pst*I sites is an *ars*1 mutation.

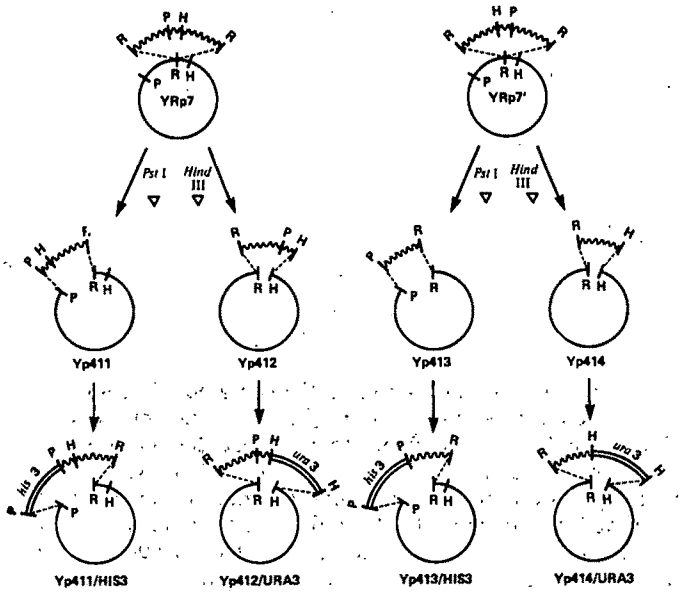


Fig. 4 Deletion analysis of Sc4101. Both *Pst*I and *Hin*dIII restriction endonucleases have unique cleavage sites in Sc4101 and pBR322. Digestion followed by circularisation and ligation (as described in Fig. 1) results in deletion of pBR322 and yeast sequences. The plasmids in the second line, derived in this manner from YRp7 and YRp7′, were identified by selecting the appropriate drug resistance after transformation of *E. coli*. Yeast structural genes were then inserted at the now unique *Pst*I or *Hin*dIII cleavage sites. The *his*3-containing yeast DNA fragment (Sc2710) was prepared by *Pst*I digestion of pGT2-Sc2605 (ref. 9) while the *ura*3 fragment (Sc2904) originated from *Hin*dIII cleavage of λ590–Sc2710 (ref. 12). The resulting plasmids shown in the second line were identified by selection for complementation of *his*B and *pyr*F lesions and for the appropriate drug resistances following transformation of the *E. coli* strains *his*B463 (ref. 28) and MB1000 (ref. 12). The structures of all of the molecules shown were confirmed by restriction endonuclease digestion and agarose gel electrophoresis. Line drawings and letterings are as described in Fig. 1 legend and text.

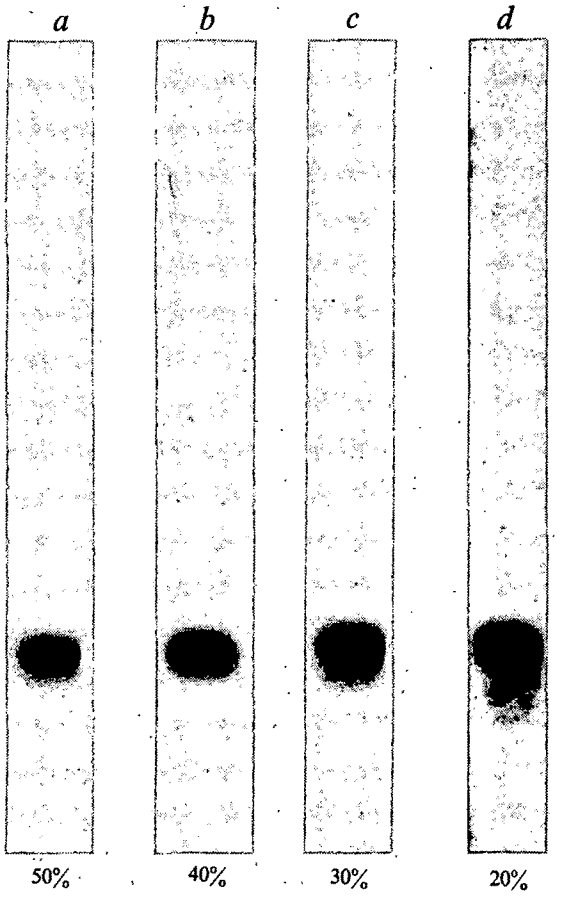


Fig. 5 Yeast sequences homologous to *ars*1. Yeast DNA was digested with *Eco*RI endonuclease and electrophoresed in a 0.6% agarose slab gel. The resulting *Eco*RI restriction spectra were transferred to nitrocellulose and then cut into four strips. After *Eco*RI digestion of YRp7 and gel electrophoresis, Sc4101 was purified by binding the DNA to a glass fibre filter (M. W. McDonnell and R.W.D., in preparation). The isolated fragment was then nick-translated. 10^6 c.p.m. of ^{32}P-labelled Sc4101 DNA was hybrised to each nitrocellulose strip in 5XSSPE (0.75 M NaCl, 50 mM $NaPO_4$, 5 mM EDTA), 0.2% SDS with 50% (v/v), 40%, 30% and 20% formamide (strips *a*, *b*, *c* and *d*, respectively). Each strip was washed in its own hybridisation conditions and autoradiographed. Long exposures were used to try to detect faint bands.

Are sequences homologous to *ars*1 found elsewhere in the yeast genome?

Using measured values for the DNA elongation rate and the total size of the yeast genome, it was estimated that a minimum of 70 initiations must occur if the cell is to replicate its DNA fully during the S phase of the cell cycle[22]. These other chromosomal replicators may have DNA sequences in common with *ars*1.

In an attempt to detect *ars*1 sequences present elsewhere in the yeast genome, ^{32}P-labelled Sc4101 DNA was hybridised to a restriction endonuclease spectrum of yeast DNA. The hybridisation conditions were varied to allow fragments with a significant proportion of mismatched base pairs to anneal. As shown in Fig. 5, even in the least stringent conditions (Fig. 5*d*), only the single homologous 1.4-kilobase pair fragment hybridised to Sc4101. The conditions used would have required at least 75% homology to *ars*1 (over a 500-base pair region) for hybridisation to occur. Alternatively, other yeast replicators probably have less than 20 sequential base pairs in common with *ars*1.

The usefulness of hybrid molecules containing *ars*1

We have previously discussed some of the uses of plasmids containing the Sc4101 fragment[9]. Results reported here point out additional applications. Vectors that contain *ars*1 (which we shall call 'YR vectors') can be maintained in yeast cells in two easily distinguishable states. Although autonomously replicating in selective conditions, YR transformants are mitotically unstable. In this state, the supercoiled YR DNA can be easily re-isolated (D.T.S., K.S. and M. Thomas, unpublished observations). In addition, stable transformants with integrated YR sequences can then be easily obtained at high frequency. In this second state, the location of the integrated DNA can be determined physically (ref. 9 and above) or by simple genetic analysis (see ref. 11). Such linkage analysis could determine the chromosomal location of a yeast DNA fragment that had been cloned in a YR vector. Integrated YR vectors can also be used for directed genetic manipulation, wherein a sequence is replaced by an altered but homologous counterpart through an integration–excision mechanism[9,12].

In addition to its uses as a vehicle for clonally isolating DNA in yeast, YRp7 will facilitate studies of yeast DNA replication in several ways. First, continuation of the preliminary genetic analysis described here will determine the sequences essential for the functioning of a eukaryotic chromosomal replicator. Second, other functions that control *ars*1 replication may be identifiable by genetic or biochemical techniques; for example, several mutants that are temperature-sensitive for the initiation of DNA synthesis have been characterised[24]; their effects on *ars*1 replication could be studied in detail. Third, the supercoiled YRp7 plasmid could be used (either with or without its chromatin protein complement) as a template for *in vitro* DNA replication systems.

The high frequency of transformation associated with *ars*1 suggests an easy method for the isolation of other chromosomal replicators. Random fragments of DNA can be inserted into a yeast vector that transforms only at low efficiency. Transformation of a yeast strain by the hybrid molecules will result in an enrichment for those fragments which allow autonomous replication. Studies of such *ars* loci isolated from yeast and other organisms are in progress.

The characterisation of a chromosomal replicator represents the first step towards the goal of constructing an operational eukaryotic chromosome. The isolation of other elements that control replication and proper segregation will not only elucidate the mechanisms of chromosome function but will provide the means for determining how chromosome structure affects gene expression.

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Ca^{2+}-sensitive gelation of actin filaments by a new protein factor

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Two protein factors which bind to, and induce gelation of, actin filaments were purified from Ehrlich tumour cells. Filamin induced Ca^{2+}-insensitive gelation, whereas a new protein factor ('actinogelin') was found to induce Ca^{2+}-sensitive gelation.

FOR a better understanding of the cellular functions of actin filaments more information on the properties of actin accessory proteins is needed. Actin-related gelation of cell-free extracts prepared from amoebae[1-3], mammalian cells[4-6] and sea urchin eggs[7] provides an excellent system for studies on actin accessory proteins, especially on the regulation of their interaction with actin filaments.

Involvement of Ca^{2+} in the regulation of microfilament function is possible, since intracellular Ca^{2+} at micromolar concentration is known to influence processes such as cell motility[8], chemotaxis[9], secretion[10,11] and fertilisation[12]. Studies on reversible inhibition of actin-related gelation by micromolar concentrations of Ca^{2+} in a large variety of cells[3,6,7-13] further support this hypothesis. Possible involvement of Ca^{2+} in HVJ(Sendai virus)-induced cell fusion[6,14-16], chemically induced cell fusion[17] and fusion of myoblast[18] has also been reported.

Ca^{2+}-insensitivity of filamin(actin binding protein)-dependent gelation

Since filamin (or a protein similar to actin binding protein of Stossel and Hartwig[4]) is concentrated in gels together with actin from Ehrlich tumour cell extracts (Fig. 1*a*, *b*), the role of filamin in Ca^{2+}-sensitive gelation was studied. As shown in Fig. 1*c*, filamin purified from Ehrlich tumour cells by a combination of the methods described for actin binding protein of macrophage[14] and for smooth muscle filamin[19,20], gave a single band by SDS-gel electrophoresis. The gel formed by mixing this protein with skeletal muscle actin consisted of only filamin and actin (Fig. 1*d*). Degree of gelation measured by pelleting of protein by centrifugation at 49,000*g* for 10 min, is dependent on the amount of filamin added but is not appreciably affected by the presence of either Ca^{2+} or EGTA (Fig. 2). Similar insensitivity towards Ca^{2+} in a purified and reconstituted system was recently reported for 'actin binding protein'-dependent gelation of rabbit alveolar macrophage extracts[21] and '57 K dalton and 220 K dalton protein'-dependent gelation of sea urchin egg extracts[22]. As a result, the search for calcium-sensitivity conferring factor(s) is under way in several laboratories[6,23,24].

Purification of a Ca^{2+}-sensitive gelation factor (actinogelin)

During a search for a factor which confers Ca^{2+}-sensitivity on filamin-related gelation of muscle actin, a protein fraction was obtained from Ehrlich cell extracts which, surprisingly, did not contain filamin but did produce gel on mixing with rabbit skeletal muscle actin, provided that 1 mM EGTA is included in the reaction mixture. No requirement for filamin was observed for gelation by this protein factor as described below. As shown in Fig. 3, the degree of gelation assayed by the centrifugation method is proportional to amounts of DE32 eluate fraction added to skeletal muscle actin. Further purification of the protein fraction on hydroxyapatite column showed that a protein, of MW 115,000 on SDS-polyacrylamide gel electrophoresis, was concentrated in an active fraction (fraction b) and the same band was also selectively concentrated with actin in gel fraction pelleted by centrifugation (Fig. 4). In contrast, there was no co-precipitation of such a band with actin from reaction mixtures containing inactive fractions (fractions a and c).

This new gelation factor was purified by the method outlined below until the product gave a single band on SDS-gel electrophoresis (Fig. 1*e*) (see legends to Figs 1 and 4 for details). Cell-free extracts were obtained by the method described previously[6] except that Tris-maleate buffer was replaced with 20 mM imidazole-HCl buffer (*p*H 7.0). After adjusting KCl concentration of the extract to 0.6 M, the extract (190 ml, about 5,700 mg protein) was fractionated by ammonium sulphate precipitation (between 15% and 35% saturation). One unit is defined as the amount of gelation factor which precipitates 1 mg of actin in a Ca^{2+}-sensitive process on centrifugation at 49,000*g* for 10 min (control experiments in which the same actin preparation was centrifuged in the same medium were performed, and the value obtained was subtracted from the experimental values). The same ammonium sulphate fractionation cycle was repeated, and the resultant fraction was dialysed and then clarified by centrifugation. The supernatant (630 mg protein, 189 U) was applied on DE32 column, and an active fraction was eluted with 0.4 KCl (77 mg protein, 215 U). Further purification was achieved by hydroxyapatite column chromatography and the active fraction (16 mg protein, 110 U) was applied on and eluted from Sepharose 6B column. On electrophoresis, about one-third of the fractions obtained (2.5 mg protein, 24 U) were judged pure (Fig. 1*e*). For higher recovery, fractions containing appreciable activity were pooled (6.2 mg protein, 57 U), and some of this was further purified by chromatography on DE32. Since filamin interferes with the assay, in crude preparations the factor cannot be determined accurately. However, a purification of ~30-fold was achieved with 30% recovery from the second ammonium sulphate fractionation step to Sepharose 6B gel filtration step. From the final yield of the protein (about 0.1% of crude extract), the content of the Ca^{2+}-sensitive gelation factor in crude extracts is calculated to be >0.3%. This protein is tentatively named as 'actinogelin' to avoid confusion with the other actin binding proteins. Gel reconstituted from skeletal muscle actin and actinogelin was found to be composed of actinogelin and actin (Fig. 1*f*).

Thus two gelation systems which differ in sensitivity towards micromolar concentrations of Ca^{2+} could be reconstituted from skeletal muscle actin and protein fractions which were purified from the same cell-free extracts. To establish whether these fractions can be detected in crude systems, SDS-gel electrophoretograms of crude cell-free extracts in the presence and absence of EGTA were compared (Fig. 1*a*, *b*). Bands corresponding to filamin, actinogelin and actin were concentrated in the gel. 'Actinogelin' and 'filamin' are confirmed by co-electrophoresis of the gel fraction with purified proteins. Protein bands of similar electrophoretic mobility (MW ~100,000–120,000 K) can be detected in published electrophoretograms of crude gel fractions of HeLa cells[25], leukocyte extracts[26] and amphibian cell extracts[13], although they were not mentioned in the texts. Thus it seems possible that two different gelation factors might co-exist in several cells other than Ehrlich tumour cells.

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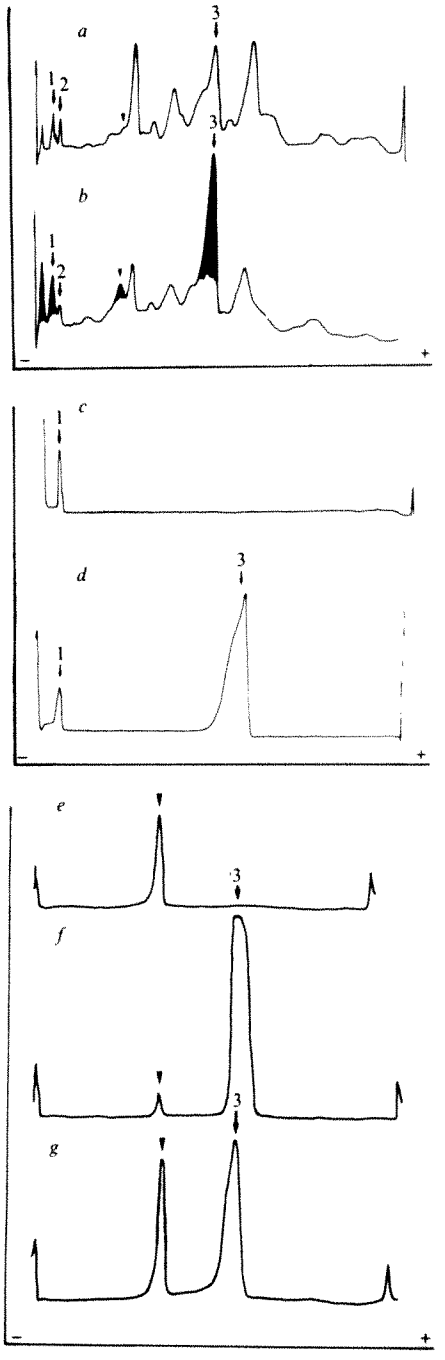


Fig. 1 SDS-polyacrylamide gel electrophoretograms of crude and purified gelation factors and gels. *a*, Crude extract; *b*, gel formed from crude extract, protein bands which were concentrated in the gel compared to the extract are shown in black. *c*, Filamin purified from Ehrlich tumour cells as described in Fig. 2 legend. *d*, Gel formed by reconstitution of 340 μg actin and 20 μg of filamin. *e*, Purified actinogelin (Sepharose 6B fraction), 10 μg. *f*, Gel formed by reconstitution of 352 μg actin and 21 μg of pure actinogelin and centrifuged at 49,000*g* for 10 min. The amount of total protein applied to the gel column was 30 μg. *g*, Precipitate formed by reconstitution of 40 μg actin and 40 μg of pure actinogelin, followed by centrifugation at 140,000*g* for 3 h. Amount of total protein applied to the gel column was 30 μg. Pure actinogelin was obtained by Sepharose 6B column chromatography of the hydroxyapatite eluate fraction. The pooled fraction obtained as described in Fig. 4 legend was concentrated to 1.0–1.5 ml and applied to a column of Sepharose 6B (1.2×95 cm) equilibrated with 20 mM Tris-HCl (*p*H 7.6) containing 50 mM sodium chloride, 0.1 μg ml^{-1} pepstatin and 1 mM sodium azide. Elution was carried out by the same buffer and 1.2-ml samples were collected. Actinogelin was eluted from the column as a major peak with some tailing material(s). The main fractions whose purity was checked by SDS-polyacrylamide gel electrophoresis and found to be homogeneous were pooled and used as pure actinogelin preparation. Bands 1, 2 and 3 correspond to filamin, myosin heavy chain and actin, respectively. Actinogelin is marked by an arrowhead. Acrylamide at 5.6% was used.

Characteristics of actinogelin-dependent gelation

The effects of free Ca^{2+} on the extent of gelation in the actinogelin-dependent system were almost identical to the effects in crude extracts—gelation was inhibited by micromolar concentrations of free Ca^{2+} (Fig. 5). Furthermore, rapid solation of the formed gel by increasing free Ca^{2+} occurred at similar Ca^{2+} concentrations in the two systems[6]. Gelation activity of purified actinogelin is similar to or higher than that of pure filamin in Ehrlich tumour cells on weight basis. Since the MW of native actinogelin is not yet known, we cannot calculate specific gelation activity. But a molar ratio of ~1 : 100 of either filamin or actinogelin (calculated as dimer) could induce detectable gelation of skeletal muscle actin.

The maximum ratio of binding of actinogelin to F-actin was estimated for a fixed amount of actin in the system (40 μg in 80 μl), with increasing amounts of actinogelin added to it, and by applying centrifugation at 140,000*g* for 3 h to precipitate F-actin. Resultant pellets were analysed by SDS-gel electrophoresis (Fig. 1*g*). A linear increase of actinogelin recovered in the pellet fractions was observed at up to 20 μg actinogelin per tube, but no further increase was detected when the actinogelin level was raised to 40 μg: no appreciable actinogelin (0–3 μg) was found in the supernatant fraction when up to 20 μg actinogelin was present in the system, whereas at 40 μg about 21 μg of the added actinogelin was recovered in the supernatant. Thus it is unlikely that the preparation is contaminated with protein which lacks actin binding ability. No measurable precipitation of actinogelin was observed in control experiments without actin. Assuming that actinogelin is mainly present as the dimer (preliminary observation), we estimate the molecular ratio of actin to actinogelin at maximum binding to be ~10–12.

Possible participation of Ca^{2+}-activated protease in Ca^{2+} inhibition of gelation[27] was checked by measuring the reversibility of the inhibition. Gelation mixture containing actinogelin and actin was preincubated in the presence of 50 μM Ca^{2+} for 60 min at 20 °C, and EGTA was added to a final concentration of 400 μM to decrease the free Ca^{2+} concentration. The degree of gelation of the treated system was identical with that of unpreincubated control. Thus, irreversible destruction of reaction components by a Ca^{2+}-activated protease could be excluded. The effect of Ca^{2+} on G to F transformation of skeletal muscle actin was measured by determining the amounts of F-actin pelleted at 140,000*g* for 3 h. No appreciable difference in the amounts of F-actin pelleted was observed between centrifugations performed in the presence or absence of Ca^{2+} in the experimental conditions used.

The possibility that small MW Ca^{2+}-binding protein similar to Ca^{2+}-dependent modulator protein[28,29] of non-muscle cells or leiotonin C of smooth muscle[30] might be present in actinogelin preparations and cause Ca^{2+}-sensitivity seems unlikely for the following reasons: (1) An actinogelin preparation purified to the Sepharose 6B step was passed through a Sephacryl S-200 column equilibrated with a medium containing 1 mM EGTA and 0.8 M KCl to facilitate dissociation of any Ca^{2+}-modulator protein that may have been present. But, no decrease in gelation activity and Ca^{2+}-sensitivity of the actinogelin preparation was observed after such treatment. (2) An actinogelin preparation was rechromatographed on DE32 in a medium containing 1 mM EDTA which was used for separation of acidic leiotonin C from leiotonin A[30], but no evidence for separation of highly acidic protein from actinogelin was obtained.

Semiquantitative densitometry of SDS-gel electrophoretograms of gel pellets showed actinogelin recover in the pellet fraction decreased in the presence of 50 μM Ca^{2+} to less than one-third of that of control with 0.5 mM EGTA. Whether this decreased binding of actinogelin to F-actin is sufficient to explain inhibition of gelation by Ca^{2+}, or whether a change in oligomeric structure such as a dimer to monomer conversion induced by Ca^{2+} must be introduced to explain the inhibition is not yet clear.

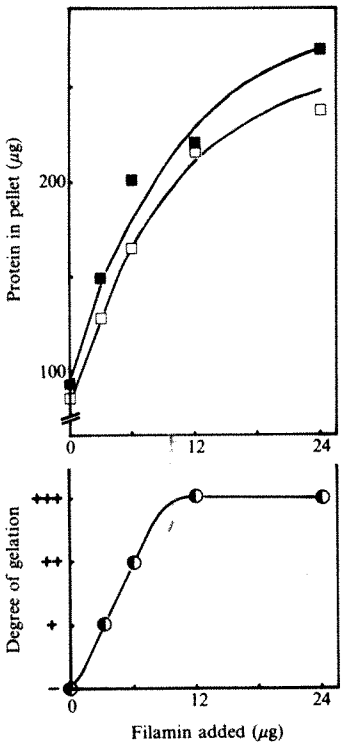


Fig. 2 Effects of Ca^{2+} on filamin (actin binding protein)-induced gelation of rabbit skeletal muscle actin. Reaction medium consisted of 10 mM imidazole-HCl buffer (*p*H 7.2), 50 mM potassium chloride, 1 mM magnesium chloride, 2.0 mg ml^{-1} of rabbit skeletal muscle actin containing ATP at a final concentration of 65 μM, and either 1.15×10^{-4} M $CaCl_2$ (■) or 5×10^{-4} M EGTA and 6.5×10^{-5} M $CaCl_2$ (□). Filamin was purified from Ehrlich tumour cells as follows. Cells resuspended in two volumes of an extraction medium containing 0.34 M sucrose, 1 mM ATP, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulphonylfluoride (PMSF), 0.1 μg ml^{-1} pepstatin, and 20 mM imidazole-HCl (*p*H 7.0 at 25 °C), were disrupted by a cell disruption bomb (Purr) as described previously[6]. The disrupted cells were centrifuged at 30,000 r.p.m. for 1.5 h in a Hitachi 55P ultracentrifuge with a RP30 rotor at 2° to 4 °C and the resultant clear supernatant was collected. As the first step, filamin was extracted from gel. After addition of 1 mM $MgCl_2$, the cell extracts were warmed to 25 °C for 1 h and then resultant gels were sedimented at 40,000*g* for 10 min in a Sorvall SS-34 rotor. The pellets were washed twice with 0.1 M KCl in 20 mM imidazole-HCl (*p*H 7.0) and resuspended in one-fifth of the original volume of a medium containing 0.6 M KCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 0.1 μg ml^{-1} pepstatin and 10 mM imidazole-HCl (*p*H 7.0). The suspension was left to stand in a cold room (4 °C) with stirring overnight to release filamin from insoluble materials. Insoluble materials containing F-actin were removed by centrifugation at 100,000*g* for 3 h at 4 °C. The supernatant was collected and subjected to the second step of purification. Saturated ammonium sulphate solution with *p*H adjusted to 7.0 was added to the supernatant to give 15% saturation. After removing the resultant precipitate, the supernatant was brought to 35% saturation by adding saturated ammonium sulphate solution. The pellet was collected and suspended in 0.6 M KI solution (containing 0.6 M KI, 20 mM $Na_2S_2O_3$, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, 0.1 μg ml^{-1} pepstatin, 10 mM imidazole-HCl *p*H 7.0). The ammonium sulphate fractionation (15–35% saturation) was repeated, and the pellet was suspended in 1–2 ml of the 0.6 M KI solution. After standing for 30 min at 4 °C, the suspension was applied to a 1.6×83 cm column of Sepharose 4B equilibrated with a low ionic strength buffer (2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, *p*H adjusted to 8.0), and eluted with the same buffer at a flow rate of 6 ml h^{-1}. Filamin was eluted as a major peak. Peak fractions were checked for purity by SDS-polyacrylamide gel electrophoresis, and fractions found to be homogeneous were pooled and used as pure filamin. If necessary, fractions with slight contamination were further purified by DEAE-cellulose (Whatman DE32) column chromatography. Filamin was eluted from the DE32 column by stepwise elution between 0.1 M and 0.15 M KCl dissolved in 20 mM Tris-HCl buffer (*p*H 7.6). Purified filamin was added to the gelation system as indicated to a final volume of 0.2 ml. Incubation was for 60 min at 20 °C. The degree of gelation was assessed by tilting tubes containing gels as described previously[6], either in the presence of Ca^{2+} or EGTA (◐). Gelation was quantitated by measuring amounts of protein pelleted by centrifugation at 49,000*g* for 10 min at 15 °C. Pellets were rinsed once by the reaction medium, then the amounts of protein were measured by the method used for the precipitate[43].

The role of actinogelin and filamin in gelation of crude extracts

Since at least two different gelation systems were found in Ehrlich tumour cell extracts, the relationship between these systems was studied. In the presence of a sub-optimum concentration of actinogelin and various amounts of filamin together with actin, gel formation is partially sensitive to Ca^{2+} at low concentrations of filamin. The extent of gelation is partially additive when EGTA is present in the medium, whereas in the presence of 50 μM Ca^{2+} it is almost identical with that of filamin alone. At higher concentrations of filamin, however, gelation becomes insensitive to Ca^{2+} (Fig. 6). Thus, the involvement of actinogelin in determining Ca^{2+}-sensitivity of the crude system cannot be unequivocally established until a quantitative determination can be made. But, quantitation of filamin and actinogelin in crude extracts is rather difficult, and effects of the other proteins which bind to actin, such as myosin and tropomyosin, on interaction of any of the gelation factors cannot be excluded. For example, heavy meromyosin inhibits sedimentability of filamin without appreciably affecting that of actin[25], and filamin was found to inhibit actin activation of heavy meromyosin ATPase[31]. Furthermore, the possible role of other regulatory systems has to be considered. Some of the studies on gelation of crude cell-free extracts which may shed light on this problem are discussed below.

Gelation of some ascites tumour cell extracts is enhanced by tryptic digestion of the extracts. Close examination of the resultant gel from the treated sample revealed that no filamin-like component is concentrated in the gel, whereas that obtained from untreated control contained a filamin-like component[32]. Although Ca^{2+}-sensitivity was not tested (EGTA was included in the reaction mixture), this result suggests the presence of a gelation factor which is different from filamin. On the other hand, Brotshi *et al.*[21] surveyed gelation factors of rabbit alveolar macrophage extracts and found that the main component of gelation of the extracts is high MW 'actin binding protein'. But, as they did not include Ca^{2+} chelators in the reaction medium, no Ca^{2+}-sensitive gelation factor would have been detected.

Thus actinogelin appears to play an important part in the Ca^{2+}-sensitive gelation of the crude extract of Ehrlich tumour cells for the following reasons: (1) In crude extracts actinogelin becomes concentrated in the gel; (2) the concentrations of Ca^{2+} required for inhibition of gelation are almost identical in crude and reconstituted systems; (3) no other fractions from the crude extract would either induce Ca^{2+}-sensitive gelation or confer Ca^{2+}-sensitivity to filamin-dependent systems; (4) the amounts of actinogelin recovered in the purified fraction were sufficient to explain gelation of the crude extracts.

Comparison of actinogelin with the other actin-accessory proteins

There are several proteins which bind to either actin filaments or monomeric actin in animal cells. Those which bind to filamentous actin and cross-link between actin filaments could form actin-related gels. Filamin (actin binding protein) can form

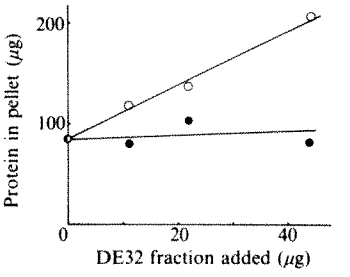
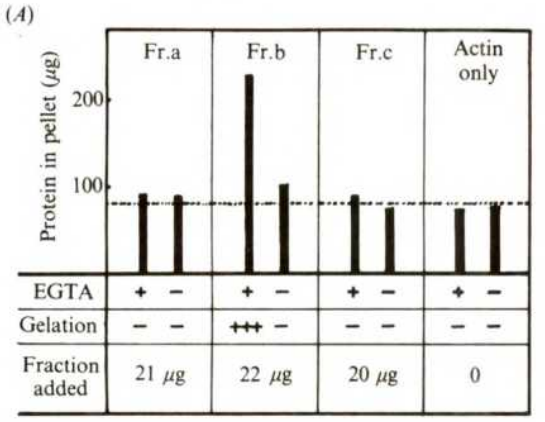


Fig. 3 Reconstitution of Ca^{2+}-sensitive gelation with rabbit skeletal muscle actin and a crude fraction partially purified from Ehrlich tumour cell extracts by DE32 chromatography. ○, In the presence of 1 mM EGTA and 9×10^{-5} M $CaCl_2$; ●, with 9×10^{-5} M $CaCl_2$ added.



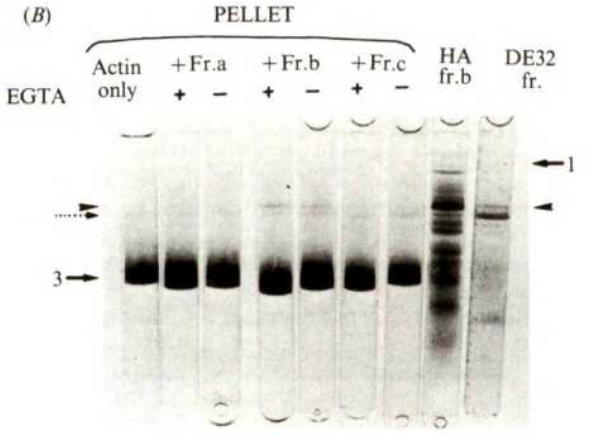


Fig. 4 Purification of actinogelin by hydroxyapatite chromatography. Actinogelin was purified to DE32 step as follows: Cell-free extracts were prepared as described in Fig. 2 legend. After adjusting KCl concentration of the extract to 0.6 M, the extract was fractionated by ammonium sulphate (between 15% and 35% saturation). The resultant pellet was dispersed with a buffer containing 10 mM imidazole-HCl (*p*H 7.0), 0.1 mM KCl, 2 mM $MgCl_2$, 0.1 mM PMSF and 0.1 μg ml^{-1} pepstatin and then dialysed overnight against the same buffer. The dialysed solution was adjusted to a final concentration of KCl of 0.6 M, and was spun at 100,000*g* for 3 h, and clear supernatant was fractionated again by ammonium sulphate between 15% and 35% saturation. The resultant pellet was dispersed with a buffer containing 20 mM imidazole-HCl (*p*H 7.2), 0.1 mM PMSF and 0.1 μg ml^{-1} pepstatin and then dialysed overnight against the same buffer. Insoluble materials were removed by centrifugation at 40,000*g* for 10 min in a Sorvall SS-34 rotor and the clear supernatant was applied to a 3 × 15 cm column of DE32 which was equilibrated with 20 mM imidazole-HCl buffer (*p*H 7.2). The column was washed with 100 ml of 20 mM imidazole buffer and subsequently washed with 400 ml of 20 mM imidazole buffer containing 0.2 M KCl, and then actinogelin was eluted with 400 ml of 0.4 M KCl in 20 mM imidazole buffer. The fractions eluted with 0.4 M KCl were dialysed against 10 mM potassium phosphate (*p*H 6.8) and applied to a 2 × 6.5 cm column of hydroxyapatite. After washing the column with 70 ml of 50 mM potassium phosphate (*p*H 6.8), elution was carried out with a linear gradient prepared with 60 ml of 50 mM potassium phosphate and 60 ml of 0.25 M potassium phosphate (*p*H 6.8) at a flow rate of 10 to 12 ml h^{-1}. Actinogelin was eluted as a major peak between 0.1 and 0.2 M potassium phosphate. Fractions with an appreciable actinogelin content on SDS-polyacrylamide gel electrophoresis were pooled as fraction b. Fractions just preceding the actinogelin peak were collected as fraction a, and those following the fraction b were used as fraction c. *a*, The gelation activity of the three fractions designated a, b and c was measured as described in Fig. 2 legend. Only fraction b has the ability to reconstitute Ca^{2+}-sensitive gelation. *b*, Electrophoretograms of purified fractions and gels. Although several protein bands are concentrated in fraction b compared with DE32 fraction, only a protein band (actinogelin) marked by an arrowhead (►) is bound to actin. The band marked no. 3 is actin and the band marked by a dotted arrow is a contaminant of this particular actin preparation. The arrow marked no. 1 shows the migration position of filamin in the conditions used. The amounts of total protein applied to each acrylamide gel column were 25, 46, 42, 43, 42, 31, 31, and 13 μg, respectively from left to right. Acrylamide gel at 5.6% was used.

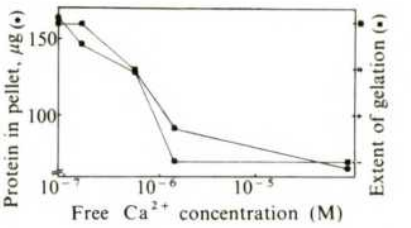


Fig. 5 Effect of free Ca^{2+} concentrations on the extent of gelation of a reconstituted system consisted of skeletal muscle actin and actinogelin. Reaction conditions were similar to those described for Fig. 2, except that 472 μg of actin and 150 μg of crude actinogelin (DE32 fraction) were used. Free Ca^{2+} concentrations were manipulated by an EGTA-Ca^{2+} buffer system[43] as described previously[6].

Ca^{2+}-insensitive gels as described above, α-actinin and tropomyosin have been shown to bind to actin cables by a fluorescence technique[33], and α-actinin produced gel when added to F-actin[34,35], although this gelation was not sensitive to Ca^{2+} (K. Maruyama, personal communication). Mixing of F-actin with tropomyosin and troponin also produced transient gelation, but this gelation was not inhibited by Ca^{2+}, although reformation of gel from high temperature-dissociated samples was inhibited by Ca^{2+} (ref. 36). The other characteristics of troponin-related gelation[37] are quite different from those of actinogelin-related gelation described in this article, and there is no evidence for the presence of troponin in non-muscle cells[38]. Kane's 58,000 MW protein isolated from sea urchin eggs is required for actin bundle formation, and interestingly, there is another protein (MW 220,000) which binds to actin–58,000 MW protein 'bundles' and is required for gel formation in sea urchin eggs[22]. No Ca^{2+}-sensitivity was found in this reconstituted system, although crude extracts of sea urchin eggs formed a Ca^{2+}-sensitive gel[7]. Lower MW proteins (gelactins) have been implicated in actin-related gelation of *Acanthamoeba castellanii* extracts[2]. Ca^{2+} or Mg^{2+} is required for this type of gelation. Condeelis and Taylor[3] have reported Ca^{2+}-sensitive gelation of cell-free extract of *Dictyostelium discoideum*, and purification of gelation factor(s) is under way[24]. It was found recently that microtubule associated proteins interact with actin filaments, although binding force between these proteins is rather weak, in other words, no co-precipitation of microtubule associated proteins with F-actin could be observed[39].

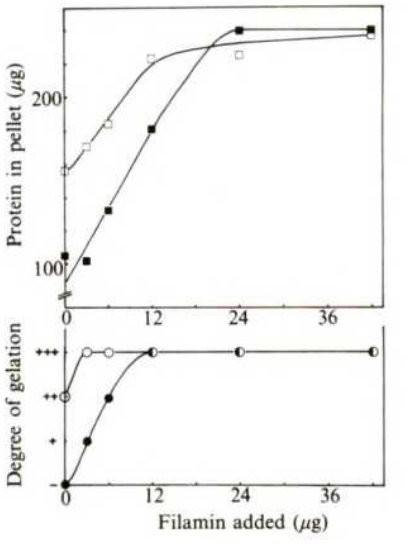


Fig. 6 Ca^{2+}-sensitivity of gelation reconstituted from skeletal muscle actin, actinogelin and filamin. Reaction conditions were similar to those described for Fig. 1, except that 400 μg of actin, 13.6 μg of actinogelin (hydroxyapatite fraction b) and the indicated amounts of filamin were used. □, ○, Experiments in the presence of 0.5 mM EGTA and 65 μM $CaCl_2$: ■, ●, in the presence of 115 μM $CaCl_2$.

Binding of a large amount of F-actin to clathrin (major protein of coated vesicles) was reported recently[40], but no effect of Ca^{2+} on this interaction is known. Thus, none of the gelation-inducing proteins reported previously has been identified as a Ca^{2+}-sensitive gelation factor, with the possible exception of a protein fraction reported in an abstract[23] which confers Ca^{2+}-sensitivity on actin binding protein-dependent gelation. The relationship between this protein and actinogelin is not yet clear, as a detailed report on Ca^{2+}-sensitivity conferring protein is not yet available. One difference is obvious, however—actinogelin alone may form a gel by interaction with F-actin, but the macrophage factor requires actin binding protein for gelation[23].

Micromolar concentrations of Ca^{2+} seem to exert at least two different effects on microfilament-dependent functions[38]; one is the well established effect of Ca^{2+} on actomyosin ATPase and superprecipitation of actomyosin[38,41] and the importance of this type of regulation is well known. The possible importance of Ca^{2+}-regulation of actin-to-actin (or actin-to-actin binding proteins) interaction has received less attention. But, as is reported or quoted here, Ca^{2+} definitely affects actin-to-actin interaction reversibly at concentrations similar to those which activate actomyosin ATPase. And, the distribution of actin is known to change extensively in several cellular functions, such as virus-induced transformation, in non-muscle cells. Thus, those two types of regulation seem to be equally important in non-muscle cells.

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letters

Universal microwave radiation and extragalactic γ radiation

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The detection of an extragalactic component of celestial γ-radiation[1,2] has stimulated the question of the possible contributions from external point sources, especially since the detection of γ rays from NGC4151 (ref. 3) and from 3C273 (ref. 4). Here we discuss the role of the inverse Compton effect (ICE) of relativistic electrons on universal microwave photons for the production of high energy γ rays from normal spiral galaxies.

It was estimated[5,6] from the total galactic γ-ray luminosity of our Galaxy that the possible contribution from normal spiral galaxies to extragalactic γ radiation is between 4 and 100% of the total for energies >100 MeV, 100% being reached if evolutionary effects within the involved spiral galaxies are taken into account[6]. The resulting flux from all involved external objects in the framework of a Friedman world model is given by[6,7]

$$I(E_\gamma) \propto H_0^{-1} \cdot \int_0^{z_0} \mathrm{d}z\, Q_g[(1+z)\cdot E_\gamma, z] \cdot (1+\Omega\cdot z)^{-1/2} \cdot (1+z)^{-1} \quad (1)$$

where H_0 is the Hubble constant, Ω is the density parameter and $Q_g(E'_\gamma, z)$ is the total γ-ray emission of one object within the ranges of energy $E'_\gamma, E'_\gamma + \mathrm{d}E'_\gamma$ and redshifts $z, z+\mathrm{d}z$, which in this calculation is restricted to γ photons from normal spiral galaxies. To simplify the estimation of this contribution all normal spirals are assumed to be of the same age, t_g^0, which is related to the value of z_0 up to which the redshift z has to be integrated by $t_g^0 = t_g(z=0)$, where the time scale is related to the scale of redshifts by

$$t_g = H_0^{-1} \cdot \int_z^{z_0} \mathrm{d}z'\,(1+z')^{-2} \cdot (1+\Omega\cdot z')^{-1/2} \quad (2)$$

Here we compare the contribution of the ICE of relativistic electrons on universal microwave photons for the production of high-energy γ rays with those from other electromagnetic processes such as bremsstrahlung of relativistic electrons. To simplify the calculations we assume that the mean γ-ray emission of spiral galaxies has a diffuse origin and that the physical parameters which are relevant to these processes are equal to those in our Galaxy and do not change as these galaxies evolve,

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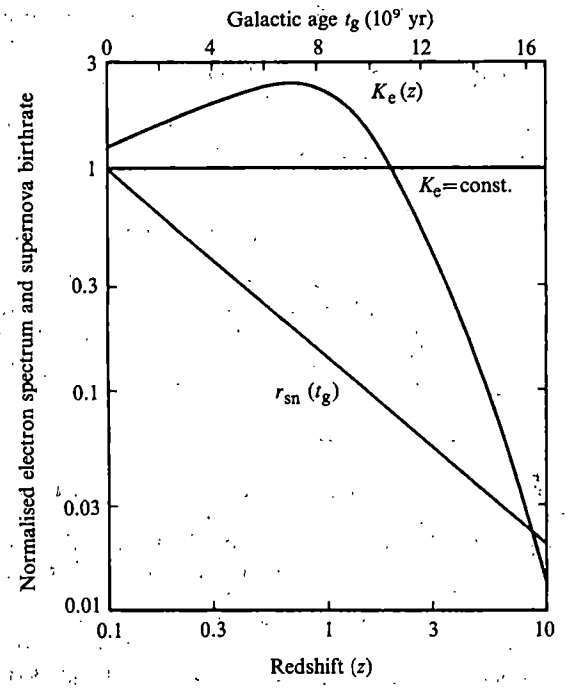


Fig. 1 Normalised supernovae birthrate $r_{SN}(t_g)$ (from ref. 10) and normalised electron spectrum $K_e(z)$ of high-energy electrons for the case of time-dependent electron sources and losses and for the case of constant galactic parameters. $\Omega = 0.08$; $H_0 = 60$ km s^{-1} Mpc^{-1}.

except for the production rate of relativistic electrons which is believed to be associated with the birthrate of supernovae or pulsars, and with the cosmological evolution of the energy distribution and temperature T of the universal microwave radiation. Consequently, the cosmological change of the electron spectrum must also be considered. At present it is not possible to investigate this problem in more detail.

The two aforementioned processes are essentially influenced by the differential energy spectrum of relativistic electrons. As we wish to consider γ rays in the energy range around 100 MeV, for bremsstrahlung the corresponding electron energies are around 200 MeV and for ICE on microwaves around 200 GeV. In these two energy ranges the electron spectrum in our Galaxy is of the form

$$I(E) \propto E^{-p} \tag{3}$$

with two different values of p (refs 8, 9). The resulting differential source functions of γ photons from electron bremsstrahlung and ICE on microwaves are

$$Q_{br}(E_\gamma) \propto E_\gamma^{-p_1} \tag{4}$$

and

$$Q_{MW}(E_\gamma) \propto E_\gamma^{-(p_2+1)/2} \cdot T^{(p_2+5)/2} \tag{5}$$

respectively, with different values of p_1 and p_2. The ratio of these two source functions in our Galaxy at $E_\gamma = 100$ MeV has been estimated as

$$r = Q_{br}(100\,\text{MeV}) : Q_{MW}(100\,\text{MeV}) \approx 40:1 \tag{6}$$

which has comparatively large uncertainties.

The temperature of the universal microwave radiation varies as $T(z) = T_0 \cdot (1+z)$ with $T_0 = 2.7$ K; the corresponding photon energy density is $w_{phot}(z) = w_{phot}(0) \cdot (1+z)^4$. Therefore this increase of temperature would be expected to increase the production of γ rays due to ICE on microwaves with increasing redshift according to equation (5). The sources of the electrons may be supernovae or pulsars with a spectrum

$$q(E, t_g) = \bar{K} \cdot E^{-s} \cdot r_{SN}(t_g)$$

where $r_{SN}(t_g)$ is the supernovae birthrate, normalised to $r_{SN}(t_g^0) = 1$. Models on galactic evolution[10] suggest that

$$r_{SN}(t_g) \propto \exp\{-t_g/t_c\} \tag{8}$$

with the characteristic time t_c of the order of 4×10^9 yr. The increase of w_{phot} with increasing redshift leads to increasing energy losses $\dot{E} \propto w_{phot} \cdot E^2$ which changes the equilibrium electron spectrum. Taking into account that the electron lifetime in our Galaxy is of the order of 10^7 yr, the time scale of the change of the supernovae birthrate is large compared with the time scale of electron diffusion. The change of the electron spectrum is thus determined by a continuity equation for the differential number density of electrons, $N(E, t_g)$, in which energy losses and leakage are balanced by a source term

$$\frac{\partial}{\partial E}(\dot{E} \cdot N(E, t_g)) + \frac{N(E, t_g)}{\tau} = \bar{K} \cdot E^{-s} \cdot r_{SN}(t_g) \tag{9}$$

with the time appearing as a parameter. At electron energies around 200 GeV which are essential for the ICE on microwaves, energy losses are dominated by synchrotron emission and inverse Compton scattering which are $\propto E^2$. Thus

$$\dot{E}(E, z) = \{c_1 + c_2 \cdot (1+z)^4\} \cdot E^2 = a(z) \cdot E^2 \tag{10}$$

The solution of equation (9) with respect to equations (8) and (10) expressed in terms of redshift, z, is

$$N(E, z) \propto f(E, z) \cdot E^{-2} \cdot \int_E^\infty \mathrm{d}\mathbf{E} \cdot \mathbf{E}^{-s} \cdot \exp\{+1/\tau \cdot a(z)\mathbf{E}\} \tag{11}$$

with

$$f(E, z) = r_{SN}(t_g(z)) \cdot a^{-1}(z) \cdot \exp\{-1/\tau \cdot a(z) \cdot E\} \tag{12}$$

At high energies this expression reduces to

$$N(E, z) = K_e(z) \cdot E^{-(s+1)} \tag{13}$$

where $K_e(z)$ is given by $\bar{K} \cdot r_{SN}(t_g(z)) \cdot a^{-1}(z) \cdot (s-1)^{-1}$. $\bar{K}$ and s are adjusted to experimental data[9] at $z = 0$. Figure 1 shows the evolution of K_e for the case of z-variable energy losses and supernovae births in comparison to the local value which is normalised to unity. Also shown is the normalised supernovae birthrate as a function of galactic age (taken from ref. 10). For the 200-MeV electrons, the main energy losses are due to ionisation and bremsstrahlung[11], so the increase of the photon energy density of the microwaves has negligible influence on these electrons. To compare the contributions to extragalactic γ radiation by the two abovementioned processes, equations (4) and (5) are inserted into equation (1) to give

$$I_{br}(E_\gamma) \propto H_0^{-1} \int_0^{z_0} \mathrm{d}z \cdot K_e'(z) \cdot E_\gamma^{-p_1} \cdot (1+z)^{-(p_1+1)} \cdot (1+\Omega z)^{-1/2} \tag{14}$$

$$I_{MW}(E_\gamma) \propto H_0^{-1} \int_0^{z_0} \mathrm{d}z\, K_e(z) \cdot E_\gamma^{-(p_2+1)/2} \cdot T_0^{(p_2+5)/2} \cdot (1+z) \cdot (1+\Omega z)^{-1/2} \tag{15}$$

The evaluation of the integrals (14) and (15) can be made for three different models. H_0 is adopted to be 60 km s^{-1} Mpc^{-1}, and Ω is taken as 0.08. The actual age t_g^0 of normal spiral galaxies and the corresponding value z_0 of redshift are not well known and need not correspond to the present observational limit of $z_{max} \sim 3.5$ for quasars. Therefore, even higher values can also be used.

Model A: both the equilibrium galactic electron spectrum and the electron sources are considered to be constant during the whole integration period. In this case the contribution of ICE on microwaves (curve A_2 in Fig. 2) overtakes the bremsstrahlung contribution (curve A_1) at a redshift of $z \sim 5$. Consequently, the major part of extragalactic γ radiation from spiral galaxies would be due to the ICE on microwaves at high redshifts.

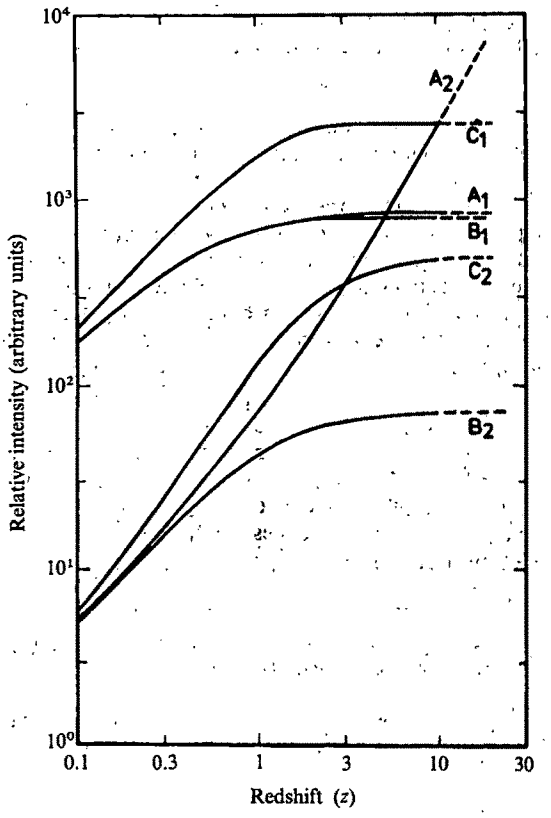


Fig. 2 Relative contributions of electron bremsstrahlung (index 1) and inverse Compton effect (index 2) from normal spiral galaxies to extragalactic γ radiation for the three evolution models A, B and C. $\Omega = 0.08$; $H_0 = 60$ km s^{-1} Mpc^{-1}; $E_\gamma = 100$ MeV.

Model B: the equilibrium electron spectrum at high energies is assumed to be dominated by energy losses $E \propto E^2$; the electron sources are still considered to remain constant. The resulting curves are B_1 and B_2. The bremsstrahlung contribution changes to a very small degree in comparison with A_1, but the ICE contribution is much lower than in model A. It shows a considerable flattening at high redshifts with a ratio $r_B = 11:1$.

Model C: electron sources as well as the equilibrium electron spectrum are assumed to be variable with galactic age. In this case the relative contributions by both processes (indicated by C_1 and C_2 in Fig. 2) increase in comparison with model B; a flattening of the curves is observed for redshifts greater than 4. Here the ratio of the contributions from the two processes at high redshifts is $r_C = 5.5:1$.

We conclude, therefore, that the contribution of γ photons from ICE on microwaves to extragalactic γ radiation is possibly more important than from local effects in our Galaxy; final results strongly depend on the evolution of the involved electron spectrum. In the case of no or weak evolution of the electron spectrum γ rays produced by ICE on microwaves can provide a considerable fraction of extragalactic γ radiation from spiral galaxies. Furthermore, the total galactic spectrum seems steeper at high redshifts than it is locally. These results depend on the choice of the cosmological parameters; if larger values for Ω are used, the integrals (14) and (15) change to a small degree.

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Is there a ring around the Sun?

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More than 300 years after the discovery of Saturn's rings, material rings or ring systems have been found in rapid succession around two of the major planets, Uranus[1] and Jupiter[2]. With rings now known to exist around three of the four major planets, it is natural to assume that they also exist around Neptune. But what about the major object in the Solar System? Is the Sun itself surrounded by a ring of material? Although the planetary system already forms such a 'ring' system, what we have in mind here is the distinct possibility of a ring of rocky material lying no more than a few solar radii from the Sun, by analogy with the spatial configuration of the three planetary ring systems now known. We summarise here the evolutionary, physical, chemical and observational astronomical constraints that can be placed on such a hypothetical ring.

Models of planetary evolution in the early solar nebula give few definite clues pointing to the mass, orbital radius or size distribution of possible objects within the orbit of Mercury. In the absence of any reliable theoretical prediction, one can use the 'Titius–Bode Law' of planetary distances as an indicator of the orbital radius of a planet (or asteroid belt). Although there are several specific functional forms of this relation[3], the one suggested by Blagg[4] offers the closest fit to both the planetary orbital radii and the planetary satellite system orbital radii. Blagg's law predicts that the next orbit inside Mercury's orbit would lie at a distance of 0.175 AU from the Sun. This distance is marginally smaller than the radius of the synchronous orbit for a planet orbiting the sun, which lies at a distance $a_{\text{synchronous}} = (GMT_\odot^2/4\pi^2)^{1/3} \simeq 0.177$ AU, assuming $T_\odot = 27$ d. Since the hypothetical planet lies within the synchronous orbit, tidal forces would tend to drag the object towards the Sun, until it breaks up at the Roche radius a_{Roche}. For fluid bodies, $a_{\text{Roche}} \simeq 2.46(\bar{\rho}_\odot/\rho_r)^{1/3} R_\odot$. For material of density $\rho_r \simeq 2$ g cm^{-3} (for example, a refractory material such as graphite) one finds $a_{\text{Roche}} \simeq 2R_\odot$ and for refractory (Ca, Al) silicates of $\rho \simeq 3.5$ g cm^{-3} the Roche limit moves inwards to $\sim 1.6R_\odot$. Such an argument naturally gives rise to a ring system about the Sun analogous to those found about the major planets. Indeed, a similar evolution has been suggested for the origin of the rings of Uranus[5,13]. It is inapplicable, however, in the present case. The time scale τ for a planet of mass m, radius A and initial orbital radius r_0 to spiral into the Sun (mass M and relative energy dissipation rate Q) by tidal drag is

$$\tau \cong \left(\frac{4}{117}\right) Q r_0^{13/2} (M/G)^{1/2} A^{-5} m^{-1} \text{ (seconds)} \qquad (1)$$

Assuming $m = 10^{28}$ g, $A = 10^9$ cm, and $Q > 1$, one finds $\tau > 10^{25}$ s, much greater than the age of the Solar System, $T = 10^{17}$ s. As we argue below, $m \ll 10^{28}$ g (at least at present), so that τ is a lower limit. Of course, this argument would not preclude the possibility that an incident body rather than a body in a slowly decaying orbit could have moved towards the Sun and broken up on a much shorter time scale. A cometary or meteoritic supply or an original refractory condensate in the intra-mercurial region is also possible.

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We know of no other evolutionary arguments which would suggest either the present position, mass or other properties of a material ring around the Sun. We therefore consider what present dynamical considerations say about any possible ring system.

A ring of particles in orbit about the Sun will give rise to a gravitational quadrupole moment, adding a source of perturbations to the planetary orbits. The most sensitive probe of such a mass quadrupole source is provided by the orbit of Mercury. Two effects are possible. First, the ring could produce an extra contribution to the advance of the perihelion of Mercury's orbit (as well as the other inner planets). The discrepancy between the observed perihelion advance, and the prediction based on newtonian gravity was originally taken as evidence for a planet or ring inside Mercury's orbit[6]. We shall assume here that general relativity is the correct theory of gravity, and take as an upper limit on any possible contribution to the perihelion advance due to the hypothetical ring the uncertainty in the relativistic contribution, that is, $\Delta\phi_{\rm ring} < 10^{-2}\ \Delta\phi_{\rm GR}$, where $\Delta\phi_{\rm GR}$ is the general relativistic contribution to the perihelion advance, equal to about 43″ per century for Mercury[7]. A straightforward calculation shows that a circular ring of particles of total mass M and orbital radius R (having width and thickness much less than R) gives rise to a perihelion advance

$$\Delta\phi_{\rm ring} = \frac{1}{4}\frac{MR^2c^2}{GM_\odot^2 a(1-e^2)}\Delta\phi_{\rm GR} \qquad (2)$$

where a and e are the semi-major axes and eccentricity of Mercury's orbit, respectively, and $\Delta\phi_{\rm GR}$ is the expected general relativistic contribution to $\Delta\phi$. (Alternatively, the ring is equivalent to a mass quadrupolar moment $J_2 = [MR^2/2]M_\odot R_\odot^2$.) With the observed limit on $\Delta\phi_{\rm ring}$ (or on J_2) we find

$$MR^2 < 4\times10^{48}\ {\rm g\ cm^2} \qquad (3)$$

Precession of the nodes of Mercury's orbit gives a limit which is about two orders of magnitude higher, assuming that the orbital plane of the ring is the same as the invariant plane of the Solar System.

We next consider the size of any hypothetical bodies. Here one is guided by the various dynamical forces acting on the bodies. The Poynting–Robertson effect drags objects in a circular orbit of radius R into the Sun on a time scale[8]

$$t \simeq 3\times10^{-20} AR^2\rho_{\rm r}\ ({\rm years}) \qquad (4)$$

where A(cm) and $\rho_{\rm r}$(g cm^{-3}) are the radius and density of the ring particles. For objects at a few solar radii and densities of a few g cm^{-3}, only kilometre-size objects have survived since the formation of the Solar System. Drag by motion through the solar wind or outer corona are of comparable importance at a few solar radii, negligible at greater distances. We have also examined the so-called Yarkovsky drag on rotating heated bodies[8] (for all angular rotation rates) and find it places a less restrictive lower limit on the allowed size of the particles.

We now consider constraints on the properties of the ring particles by the requirement of survival against solar irradiation. For most materials, the direct effect of sputtering by the solar particle beams will ablate away only a few centimetres of almost any material per 10^9 years. (Sputtering will, however, considerably darken the surface materials and redden the reflected light, making visual observation less likely—see ref. 9.) The fierce solar radiation field will directly heat the bodies, leading to rapid evaporation. Objects at orbital radius R, with radiation absorption coefficient α will be heated to an assumed uniform surface temperature

$$T = \left(\frac{\alpha R_\odot^2}{4R^2}\right)^{1/4} T_\odot \qquad (5)$$

For a very dark material like carbon with $\alpha \simeq 1$, the black-body equilibrium temperature at $4R_\odot$ is $\sim$2,000 K; for more reflective refractory materials (for example alumina, calcium-aluminium silicate) with $\alpha \simeq 0.1$, $T_{\rm eq} \gtrsim 1{,}100$ K. To find the vaporisation rate produced at this temperature, we must know the vapour pressure observed or computed for the assumed material composition of the bodies as a function of temperature. No simple analytical expression exists for the vapour pressure as a function of temperature for most materials. Using present numerical data for the vapour pressure as a function of temperature $p = p(T)$, we estimated the vaporisation rate for a variety of materials[10,11] ($F(T) = p(T)\ (kT/m_{\rm atom})^{-1/2}$ cm^{-2} s^{-1}, where $m_{\rm atom}$ as the atomic mass of the vaporising species). Carbon seems to be both abundant and refractory enough to survive in the near solar environment. Graphite seems the likely form. Lewis and Ney[12] have recently examined the conditions for the condensation of refractory grains in circumstellar envelopes and found that for a broad range of C/O ratios, Fe and Fe_3C condense stably and permit graphite formation over a wide range of vapour pressures below 2,000 K.

Combining the minimum size restrictions set by the Poynting–Robertson effect with the vaporisation by solar irradiation, we find that graphite bodies (absorption coefficient $\alpha \simeq 1$, density $\rho \simeq 2$ g cm^{-3}) of radius $A \gtrsim 10$ km could survive both vaporisation and drag for the age of the Solar System, but only at a distance of about $4R_\odot$. At a distance of only $2R_\odot$, even an object as large as is allowed by considerations of possible tensile strengths ($A \simeq 300$ km) would evaporate, no matter what its composition, in less than 10^9 years.

Finally, we look at the possible destruction of the ring by particle collisions. There are two relevant time scales for the collisional evolution of rotating ring particles. First, there is a relatively short time scale for ring flattening and circularisation of orbits. A straightforward analytical calculation gives for this time scale $t_1 = (t_{\rm orbit}/\pi N)(R/A)^2$, where $t_{\rm orbit}$ is the orbital period at heliocentric distance R and N is the number of ring particles. For $N \simeq 10^6$, $R \simeq 4R_\odot$ and $A \simeq 10$ km, $t_{\rm orbit} \simeq 10^5$ s and $t_1 \simeq 10^2$ yr. After this time, the only particles surviving collisional breakup or particle accumulation are those in circular orbits. The disrupted particles are irrelevant for the present considerations, and we are only concerned with the remaining particles in circular orbits. Detailed numerical calculations[14,15], however, show that the ring subsequently disperses on a much longer time scale $t_2 \simeq (t_{\rm orbit}/3N)(R/A)^4$. For the ring parameters derived above, one has $t_2 = 10^{13}$ yr. Thus, an initially present or later formed ring of 10^6 particles in circular orbits will be stable over the age of the Solar System.

We therefore arrive at a consistent picture of the most massive primordial ring allowed to reside around the Sun. It must lie at a distance $R \gtrsim 4R_\odot$. Its total mass is, therefore $M \lesssim 6\times10^{25}$ g. It could consist in that orbit of at most $N \lesssim 10^6$ objects of minimum radius $A \simeq 10$ km.

Would such objects be visible optically? If they consisted of graphite or other dark refractory materials with an optical albedo of a few per cent, they might be individually detectable with a visual magnitude of $m_v \cong +8$ to $+10$. Such objects would be observable, in practice, only during a total eclipse of the Sun. Their existence does not yet seem to be ruled out by any direct observations[16].

Finally, note that there is one intriguing piece of observational data which may hint at the existence of such a ring, precisely at the minimal allowable radius we derive above. Several IR observations[17,18] at 2.2 μm and at longer wavelengths find an excess emission peak at $4R_\odot$. This peak is not implausibly interpreted as resulting from vaporisation of small silicate grains continually spiralling into the Sun under Poynting-Robertson drag, but we can speculate that the excess may in part be due to a ring of larger bodies in orbit close to the Sun.

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Is SS433 a young binary neutron star?

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The highly variable X-ray and radio star SS433 has received much attention[1–3] because of its intense emission lines of oscillating frequency[4,5]. These comparatively narrow lines have been identified as two sets of Balmer and He I lines whose redshifts (and blueshifts) vary sinusoidally, according to

$$\lambda_\pm/\lambda_0 = 1.039\{1 \pm [0.0765 \cos \omega t + 0.0544]\} \quad (1)$$

with a probable period[5,6] (161.7±0.3) d, as well as a more intense 'stationary' system. Sharp subcomponents of the 'stationary' system vary in redshift[7] with a period of (13±0.1)d and radial velocity amplitude $K = (76 \pm 7)$ km s^{-1}. This same periodicity probably shows up[8] in the total optical intensity, with an amplitude of some 0.3 mag. I propose here that the moving lines are the result of stimulated emission from the inner edge of an accretion disk around a rapidly spinning neutron star, pumped by cyclotron radiation. The neutron star is in an eccentric orbit of period 13 d around the Of star SS433, and is forced by its accretion disk to precess with a period of 162 d. The 'stationary' emission line system consists of reprocessed starlight from the outer accretion disk, and the X rays together with light from the two oscillating line systems cool the inner edge of the disk which cuts deeply into the co-rotating magnetosphere of the (inclined) neutron star.

The huge red- and blueshifts of the moving emission lines imply that bulk matter is moving with velocities reaching or exceeding one sixth of the speed of light. They have often been interpreted[1] as coming from two antipodal beams; but there are enormous difficulties in producing a pair of almost relativistic, narrow, monoenergetic, and sufficiently dense beams on planetary-system-scales. If, instead, the lines are emitted by matter orbiting around a compact object[2], only their narrowness and sine-shaped law of motion are unexpected. But consider a neutron star as the likely[9] companion of the Of star SS433, whose spin is inclined at an angle χ w.r.t. the normal to the orbit plane and whose magnetic dipole moment is almost at right angles[9] w.r.t. its spin; once matter is transferred from the primary to the neutron star, at a rate exceeding the Eddington rate, it is likely to form an accretion disk[10] in the orbital plane. This disk will cut into the magnetosphere, in the shape of a thin blade[11,12]. Magnetospheric braking will slow down its innermost part to slightly sub-keplerian velocities until magnetic support comes weakly into play. The torque T exerted by the asymmetrically confined dipole field on the neutron star has been calculated by Aly[11]. Averaged over one revolution period

$$T = 2D^2 \sin 2\chi / 3\pi R^3 \quad (2)$$

where D is the magnetic dipole moment and R the radius of the disk's inner edge, and forces the spin to precess around the normal to the disk plane with a period

$$P_{\rm prec} = I\Omega/T = 1\ {\rm yr}\ I_{44.5}\Omega_2 R_7^3 B_{12.5}^{-2}/(2 \sin 2\chi) \quad (3)$$

where I is the moment of inertia, Ω the angular velocity, and B the polar surface field strength. The observed period $P_{\rm prec} = 162$ d requires

$$I_{44.5}\Omega_2 R_7^3 B_{12.5}^{-2}/(2 \sin 2\chi) = 0.44$$

a plausible value, as argued below.

The torque T, when acting on the inner edge of the disk, warps its shape such that orbiting matter acquires a velocity component perpendicular to it which peaks in magnitude where the dipole moment periodically intersects the disk. Radiation emitted at this longitude in near-keplerian motion is seen redshifted, at infinity of a Schwarzschild spacetime, by an amount given by

$$\lambda_\pm/\lambda_0 \approx (1-3u/2)^{-1/2}\{1 \pm (u/2)^{1/2}(1-u)^{-1/2} \times [\sin i \cos \omega t + \cos i\, {\rm d}\theta/{\rm d}\phi]\} \quad (4)$$

Here $u := r_S/r = 2GM/rc^2$ is the inverse radial distance in units of the Schwarzschild radius r_S, i is the inclination angle, ω the orbital angular velocity, ${\rm d}\theta/{\rm d}\phi$ measures the out-of-plane velocity component, and second order effects in u (like the general relativistic light bending effect) have been ignored. A comparison with the observed law of equation (1) yields: $u = 0.05$, $i = 28°$, ${\rm d}\theta/{\rm d}\phi = 0.38$.

In comparing equations (1) and (4), I have assumed that the moving emission lines of SS433 come from the two antipodal spots at the inner edge of an accretion disk where it is most warped, so that the spots move with the precession of the neutron star's spin. For a 1.4 $M_\odot$ neutron star, the value $u = 0.05$ corresponds to the radial distance $R = 20r_S = 0.8 \times 10^7$ cm which has been anticipated in equation (3). Here the (confined) magnetic field strength is of the order of 10^{10} G (if of the order of 3×10^{12} G at the polar caps).

Can such tiny spots emit more than $3L_\odot$ in the form of Balmer lines? For incoherent radiation, the observed intensities would imply, at temperature T, a radiating area in excess of $(10^{12}T_4^{-1/2}\ {\rm cm})^2$. Velocity broadening of the lines is <10% of their velocity shift, hence in the present model the size of the radiating spots must be $<(R/10)^2$ each, and lasing must result in an enhancement by factors of the order of $10^8 T_8^{-1}$. Note, however, that coherent radiation from an antipodal pair of spots at the inner edge of an accretion disk offers plausible explanations for the following observations:

(1) the two moving line systems often show mirror-image symmetry;

(2) sharp lines are more easily produced by a coherent mechanism than by an incoherent one, as well as rapid time variability;

(3) observed deviations from the strict redshift law in equation (1), on the time scale of days, can be understood as being produced by matter sinking more deeply towards the neutron star's surface (and eventually being accreted).

Before discussing the hypothesised laser in more detail, the overall features of this model should be considered. A binary orbital period of 13 d corresponds to a semi-major axis

$$a = (GM/\omega^2)^{1/3} = 5 \times 10^{12}\ {\rm cm}\ (M/30M_\odot)^{1/3} \quad (5)$$

$M := M_1 + M_2$, and to orbital velocities

$$v_1 \leqslant 13\ {\rm km\ s^{-1}} (M/30M_\odot)^{-2/3} \left(\frac{1+\varepsilon}{1-\varepsilon}\right)^{1/2} \quad (6)$$

$$v_2 \sin i = (M_1/M_2) v_1 \sin i \leqslant 1.3 \times 10^2 \quad {\rm km\ s^{-1}}$$

where ε = numerical eccentricity, and $\sin i = 0.48$ has been

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taken from above. The close separation of $a = 0.3$ AU is consistent with Roche lobe overflow from the primary (whose mass is assumed to be $\sim 28.6 M_\odot$), and with a strong illumination of the accretion disk. The small velocity amplitude of the primary is consistent with the observational constraint[3] $v_1 < 50$ km s^{-1}. An observed $K = 76 \pm 7$ km s^{-1} for the motion of a secondary object is consistent with our $v_2 \sin i \leq 130$ km s^{-1} because the strongest subcomponents will be emitted from the near side of the accretion disk which rotates in a retrograde sense. A variation in the total optical luminosity is explained by a variable binary separation, that is, by a variable illumination of the disk. The disk is probably transparent in the optical continuum but opaque in the UV, IR and optical lines, and retarded cooling would explain an asymmetrical light curve. Note that according to Press *et al.*[13], a substantial orbital eccentricity is expected to survive for some 10^{10} yr at the calculated separation. In this picture, the disk should also be identified with the large and variable IR source discussed by Giles *et al.*[14]. Finally, the relative phases, during the 13-d cycle, of the maxima in redshift[7] and intensity[8] can be explained by a substantial angle between the line of apsides and the line of nodes.

What is the structure of the (inner edge of the) disk? If confined by a 10^{10} G magnetic field, its internal pressure must be $> p = 10^{18.6}$ dyn cm^{-2}, corresponding to a particle density $n = p/kT \geq 10^{28.6} T_6^{-1}$ cm^{-3}. This pressure is higher than typical values for accretion disks (see refs 15, 16), probably because a high spin rate of the neutron star hampers accretion so that matter piles up and settles deeper into the magnetosphere. The inner edge of the disk is heated by interaction with the rapidly time-varying magnetic field, in addition to internal dissipation, and cooled by incoherent X-ray emission[17] and coherent line radiation. A total radiation power $L \geq 3 \times 10^{35}$ erg s^{-1} emitted near $r = 10^7$ cm implies an effective temperature $T_{\rm eff} \approx 10^6$ K, but X-ray energies ≥ 10 keV indicate the presence of a much hotter corona, with electron temperatures in excess of 10^8 K. In these conditions (with LTE assumed), the Saha equation yields a (maximum) neutral abundance fraction

$$\gamma = 4 \times 10^{-8} h_{20} T_8^{-3/2} \tag{7}$$

so that the corona is highly ionised. The scale height h is given by

$$h = (kTr^3/GM_* M_{\rm p})^{1/2} = 3 \times 10^5 \text{ cm } T_8^{1/2} r_7^{3/2} \tag{8}$$

where M_* is the mass of the neutron star, and $M_{\rm p}$ the proton mass.

Assuming that the dissipative interaction between disk and rotating magnet leads predominantly to cyclotron radiation, whose power

$$L = N\sigma_{\rm T} c B^2/4\pi = 2 \times 10^5 N B_{12}^2 \quad \text{erg s}^{-1} \tag{9}$$

should equal the total emission, the presence of $N \geq 10^{30}$ electrons is implied ($\sigma_{\rm T}$ = Thomson cross section). This radiation is emitted at frequencies $\nu_0 = eB/2\pi m_0 c = 3 \times 10^{16}$ Hz B_{12} and its higher harmonics $\nu_n = (n+1)\nu_0$ caused by a marginally relativistic beaming. The corresponding brightness temperatures

$$\begin{aligned} T_{\rm b} &\approx 2\pi^2 m_0 c^2 e (\int n \, {\rm d}s)(\nu/\Delta\nu)\beta_\perp^{2n}/3B(n+1)^3 k \\ &= 2 \times 10^6 {\rm K} (\int n \, {\rm d}s)_{14} (\nu/\Delta\nu)_1 \beta_\perp^{2n} B_{12}^{-1} (n+1)^{-3} \end{aligned} \tag{10}$$

exceed 10^6 K up to some $n (\geq 0)$ for almost relativistic electron velocities $\mathbf{v} = c\boldsymbol{\beta}$ and a magnetised column density $\int n \, {\rm d}s \gg 10^{13}$ cm^{-2}. This UV radiation floods the inner disk. It ionises the diskal gas, and is partially inverse-Compton scattered to X-ray energies in the hot corona. It is preferentially generated where the disk is most warped, leading to the highest brightness temperatures, to the highest ionisation rates, and to stimulated emission of all the lines of the neutral component. As absorption cross-sections in the lines exceed 10^{-17} cm^2, the lasing photosphere is extremely thin. Note that the absence of observable pressure broadening implies $T \geq 10^6 K n_{21}^{2/3}$, and that the absence of magnetic signature in the lines (shifts or circular polarisation[4]) implies magnetic field strengths either below 10^6 G or above 10^{10} G. The latter is probably realised. (The former alternative could hold were a non-magnetised photon cushion separating the magnetosphere from the—probably similarly magnetised—central disk.)

Why does SS433 look so different from other binary X-ray sources which involve a neutron star? It may be due to the neutron star's high spin rate, preventing rapid accretion. In the absence of a complete solution[16], one may argue that a low or vanishing accretion rate from a near disk requires a high enough angular velocity of the star, such that the (neutral) force balance radius $r_{\rm fb} = (GM_*/\Omega^2)^{1/3}$ does not greatly exceed the extent $r_{\rm cf}$ of the closed field line region. For an inclined (static) dipole field and a perfectly conducting infinitely thin 'disk', of inner radius R, the ratio $\xi := r_{\rm cf}/R$ obeys[11,12]

$$\xi = [1 + (2tg\chi/\pi \cos\phi)^2]^{1/2}, \tag{11}$$

where χ is the elevation angle of the dipole and ϕ its azimuthal angle. In my model, χ ranges twice from 0° to some maximum angle during one cycle, whence $\xi \geq 1$, and the condition $r_{\rm fb} \leq r_{\rm cf}$ implies

$$\Omega \geq [GM_*/(\xi R)^3]^{1/2} \approx 6 \times 10^2 \xi^{-3/2} s^{-1} \tag{12}$$

This estimate is unrealistically high, and suggests that the above criterion is too strict in a non-static situation. In any case, the neutron star's spin rate may be very high, comparable with that of the Vela pulsar, and its age correspondingly low, $\leq 10^4$ yr; see ref. 10.

For such a low age, one should still see the supernova remnant that was blown off at the neutron star's birth. An association with the remnant W 50 has been suggested[19].

Finally we should consider the radio structure of SS433. If the secondary involves a rapidly rotating neutron star, there should be an open field line region connecting its polar caps to its wind zone and along which a pulsar wind is blown out. This relativistic wind must intersect the primary's ordinary stellar wind, and give rise to an extended radio structure that shares both the 13-d and the 162-d period. As the pulsar wind will be orientated w.r.t. the neutron star's spin axis, it will illuminate the primary at a variable rate during its 13-d orbital and 162-d precession cycle, thus giving rise to a variable brightness of SS433, as observed[6].

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Dielectric breakdown by electrically induced chemical decomposition

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Experimental observations and our interpretation of electrical current instabilities in single crystals of ionic metal azides are described here. The subsequent insulation breakdown occurs over wide ranges of fields and delay times. The fields, at room temperature, are of the order MV m^{-1} or lower, and are 2–3 orders of magnitude smaller than the usual dielectric strength found in a homogeneous bulk solid. The breakdown is strongly time dependent and the delay after the application of the field can be up to a few days. We suggest for this low-field long-delay breakdown an 'electrochemical' mechanism which seems not to have been envisaged before.

Thoma[1] attributed breakdowns which exhibit extremely long delay times to the growth of conduction filaments due to dislocation structure. Narayan *et al.*[2] observed that breakdown in MgO single crystals at 1,300 K occurred in a field of 0.1 MV m^{-1} after >100 h. They proposed that during this incubation period the properties of the materials in the vicinity of the dislocations and small-angle grain boundaries changed. The changes increased the conductance along these pathways, leading to high conductivity filaments, local heating and dielectric breakdown. However, they did not specify the nature of the structural and electrical changes, nor the underlying mechanisms. We have set up a more detailed model for the breakdown in metal azides, but here we will, after summarising the empirical observations, describe only briefly our proposed scheme.

Figure 1 illustrates the behaviour of the current conducted by silver azide; the cases of thallous, lead and sodium azides have been found to be similar. At low fields, the conduction is by mobile interstitial Ag^+ according to previous work on polycrystalline samples[3,4]. We ascribe the decay of current with time to ionic polarisation at the electrodes, and this is supported by the frequency dependence of a.c. conductivity[5].

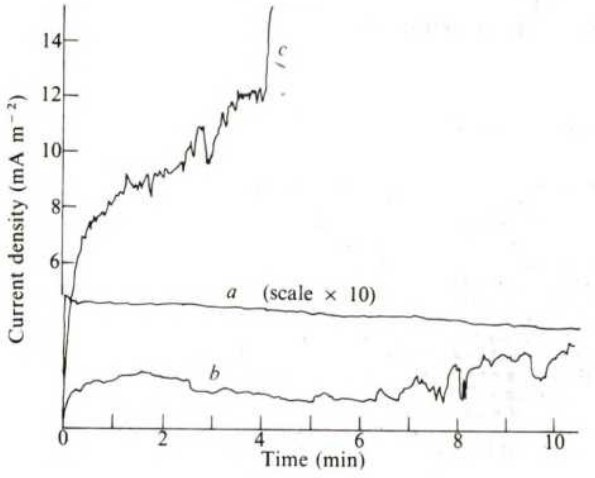


Fig. 1 Current conducted along the *c*-axis of a AgN_3 crystal at a field of *a*, 3.3; *b*, 13 and *c*, 67 kV m^{-1}, in ultrahigh vacuum, and with tantalum pressure-contacted electrodes; qualitatively the same behaviour is observed with silver and graphite electrodes.

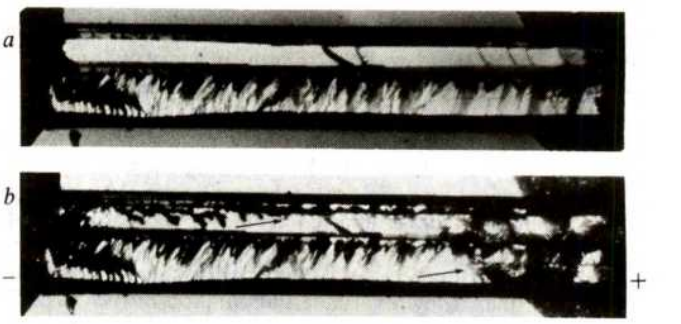


Fig. 2 Photomicrographs of a AgN_3 crystal: *a*, before; and *b*, 5 min after being subjected to 130 kV m^{-1}, arrows indicate new features formed ×95.

At moderate fields >15 kV m^{-1} (400, 800 and 200 kV m^{-1} in the cases of TlN_3, $Pb(N_3)_2$ and NaN_3 respectively), the current displays large-amplitude non-periodic fluctuations; its average value also increases with time, and at a faster rate when the field is stronger. An estimation of the interfacial fields at the polarised contacts[5] suggests that the fluctuations are caused by the double injection of electrons and holes into the crystal. We believe that the continuous increase in the current is caused by the formation of metallic filament-like films on the crystal surface; such electrical behaviour has been shown to exist for the case of ultra-thin discontinuous metal films growing on insulating substrates[6,7].

In these fields, silver and lead azides have been shown[8] to give off nitrogen at rates much greater than those corresponding to the electrolysis of the crystals as estimated from their ionic conductivities. Based on a comparison with the mechanism of thermal decomposition of silver azide[9], we propose the following scheme of elementary steps for the production of surface films. Electrons injected into the conduction band of the insulator constitute a non-equilibrium concentration and so, in their drift towards the anode, a finite number of them may be localised at the electron traps. An interstitial cation may then get discharged at a filled trap, which is thereby reset. On the surface, neutral atoms migrate (see, for example, the case of silver cyanamide[10]) to form aggregates so that the discharge is irreversible. The agglomeration gives rise to, first, a network of thin films and, at later stages, clusters of discrete particles on the surface. The appearance of such metal pebbles has been confirmed (Figs 2 and 3); energy dispersive X-ray analyses have shown them to be silver. (See also ref. 9 for transmission electron micrographs of 'pebbles' formed by thermal decomposition.)



Fig. 3 Electron micrograph of crystal in Fig. 2*b*: surface features are resolved into 'pebbles' ×1,350.

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We found that if, during the initial stage of current increase the field was removed, the conductivity returned to its original value. The removal of the field will very quickly stop further generation of silver on the crystal surface, but the accretion of metal atoms in the films at the ambient temperature will continue to change their network structure; the 'pebbles' are permanent, but their number at this stage is small. This means that the number density of the films will continue to decrease with time, and after a sufficiently long time the conductivity will return to the original bulk value. In the accompanying process of field injection of holes, valence electrons are extracted into the anode. The holes drift towards the cathode, some of them combining bimolecularly on the way: $N_3 + N_3 \rightarrow 3N_2$. This process maintains stoichiometry, and leads to the formation and evolution of nitrogen away from the anode[11].

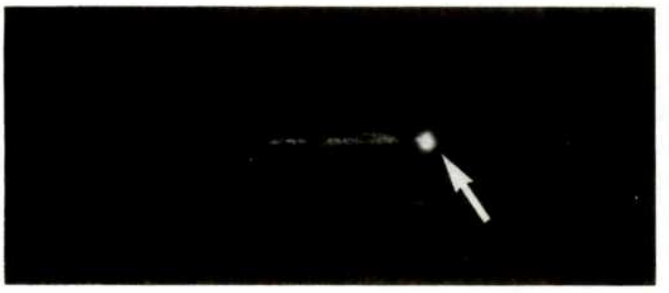


Fig. 4 A AgN_3 crystal (1.35 mm long) supporting a corona discharge (arrowed) without dielectric breakdown.

The dielectric breakdown is identified as the current runaway arising from the growth of the metal-like films in number and in size. It is therefore expected to occur after an interval of time that is longer for a lower field, when the production rate of metal atoms is slower, provided the field exceeds the limiting value for the onset of bipolar injection. Indeed we have shown that, for silver azide, the time to breakdown[5] is <1 s in a d.c. field of 400 kV m^{-1} or greater, increasing to 5 days at 15 kV m^{-1}; the other three azides show a similar wide range of breakdown times. Also, when the voltage was applied through only one conducting contact no breakdown was observed after many minutes at an estimated field of 300 kV m^{-1} (crystal in air: Fig. 4) or 1 MV m^{-1} (crystal in oil), indicating that the injection of non-equilibrium carriers is necessary. A similar result was obtained in the case of lead azide insulated with Mylar at both contacts[12]. Our proposed mechanism predicts that post-breakdown disruptions (due to localised heating by the electronic current in the film network) may start away from either electrode, a fact we have experimentally demonstrated[5] but which cannot easily be explained in alternative models previously put forward by other authors[13].

Finally, we suggest that breakdowns of extended delay times may be brought about by metallic conduction caused by certain 'electrical decomposition' processes in some ionic metal compounds besides the azides, such as halides, iodides, oxides and hydrides.

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Abnormal characteristics in crystallisation of $Ca(PO_3)_2$ glass

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Although glass is known not to crystallise below the glass transition temperature (T_g), some block specimens of metaphosphate glasses such as $Ca(PO_3)_2$, $Sr(PO_3)_2$ and $Ba(PO_3)_2$ have been found to crystallise easily at temperatures considerably lower than T_g; however, thin films of the glasses do not exhibit this behaviour[1–3]. The intrinsic characteristics of $Ca(PO_3)_2$ considered here suggest that under stress the rate of crystal growth depends on thickness, and that long polycrystalline fibres may be produced in which the crystals are orientated towards the direction of elongation.

When the chemical composition of a glass is the same as that of the growing crystal, equation (1) is applicable for most glasses[4],

$$u\eta/\Delta T \approx 0.1 \quad (1)$$

where u is crystal growth rate (cm s^{-1}), η is viscosity (P), and ΔT is the degree of undercooling. In $Ca(PO_3)_2$ glass, the crystalline phase has the same chemical composition as that of the glass. Hence, u is calculated from equation (1) to be $\approx 10^{-7}$ μm min^{-1} or several micrometres per 100 yr at T_g (melting point of β-$Ca(PO_3)_2$ = 980 °C, $T_g \approx 500$ °C, $\eta \approx 10^{14}$ P). This calculated rate of crystal growth is almost the same as that obtained by extapolating the experimental data[1] for the thin film at temperatures higher than T_g. Thus, the crystal growth rate (u) of the thin film holds for equation (1), showing the usual crystallisation mechanism. However, u of the block specimen of $Ca(PO_3)_2$ glass is extraordinarily large reaching 10^7–10^8 times that of the thin film at temperatures near T_g, thus showing an unusual crystallisation mechanism. This abnormally high rate of

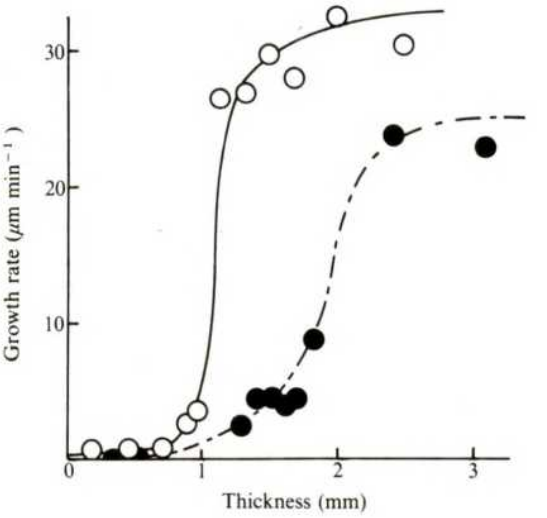


Fig. 1 Dependence of the crystal growth rate on the thickness of the $Ca(PO_3)_2$ glass plate specimen. ○, 560 °C; ●, 540 °C.

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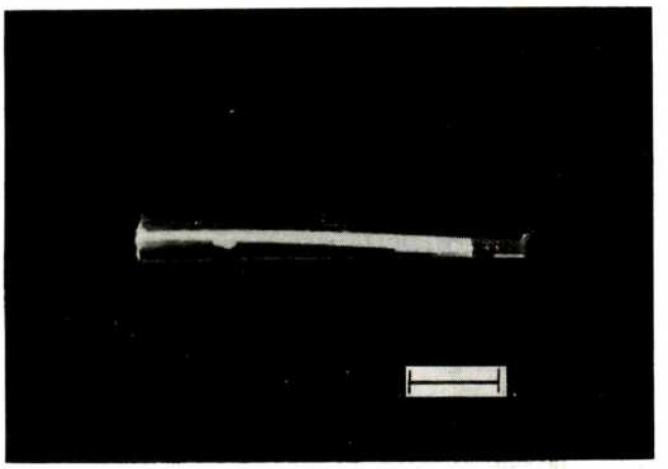


Fig. 2 Photo of orientated crystallisation in an elongated $Ca(PO_3)_2$ glass rod. Elongation, 300% at 540 °C; Heat treatment for the crystallisation, 510 °C for 6 h. Scale bar, 5 mm.

crystal growth for block specimen of $Ca(PO_3)_2$ glass has been explained in terms of induced tension in the molecular chains at the glass–crystal interface, where tension is induced by a volume difference between the crystalline and glassy phases[1].

These results suggest that the crystal growth rate depends on the shape or thickness of the glass specimen. Figure 1 shows the dependence of the rate of hemispherulitic crystal growth on the thickness of $Ca(PO_3)_2$ glass plate specimen. Abrupt change in the growth rate occurs at ≈1 mm thickness at 560 °C and ≈2 mm thickness at 540 °C, respectively, the critical thickness at which these abrupt changes take place becoming larger as the temperature decreases. I believe that this critical thickness is determined by competition between the rate of relaxation of the induced tension in the molecular chains at a growing crystal–glass interface and the rate of the crystal growth (assumed to be without stress relaxation) at a given temperature.

Another characteristic crystallisation property for $Ca(PO_3)_2$ glass is crystallisation orientated towards the direction of tension. A fine glass rod (3–4 mm in diameter) was produced from a $Ca(PO_3)_2$ glass rod held at 540°C (a little higher than T_g) by pulling and quenching under a force of 8 kg cm^{-2} to become three times the length of the original rod. This specimen was heated at 510 °C for 6 h under no load, resulting in orientated crystallisation towards the direction of elongation of the specimen; note that although the crystallisation occurred preferentially inside the glass rod, the surface of the specimen remained uncrystallised (Fig. 2). When not elongated, hemispherulite only usually grows at arbitrary places of the glass surface[1,2]; therefore orientated crystallisation must be due to stretching of the glass specimen which results in orientation of molecular chains in the glass towards the elongation direction. At temperatures around T_g, although the viscosity is low enough to allow the glass to be stretched by a sufficiently large tensile stress, the mobility of the molecular chains or chain segments is not high enough for the orientation produced by stretching to be lost during the thermal treatment to which the glass is subjected. The crystal growth rate towards the elongation direction is very high, reaching at least 10 times the growth rate of hemispherulite—usually grown from the block specimen of the glass prepared with no applied stress, the growth rate depending on conditions for the preparation of the fine rod (elongation times, elongation temperatures, rate of quenching).

It is surprising that $Ca(PO_3)_2$ glass exhibits intrinsic crystallisation below T_g, that the crystal growth rate depends on the thickness, and that the crystallisation may be orientated under tension. These phenomena have not yet been found in inorganic glasses. I believe that these crystallisation characteristics are due to structural features in that $Ca(PO_3)_2$ is comprised of molecular chains in both the glass and crystal. We can expect unusual behaviour in this type of glass that has never been observed in a glass network structure such as commercial industrial glasses.

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$^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios in magmatic gases from ridge volcanism in Afar

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Most data on the stable isotope composition of deep-seated carbon and sulphur come from igneous rocks and low-temperature gases. Very little information comes from high-temperature gases which escape from magmas ($T = 1{,}000 \pm 200$ °C), even though these are the best specimens of primary igneous fluids. Moreover, the only available $^{13}C/^{12}C$ data on carbon in high-temperature basaltic gases[2–4] are largely divergent: low ratios from −14 to −26‰ versus PDB were measured in gases from Hawaii[2,3,5], which is consistent with the values for reduced carbon in basalts[6], whereas higher ratios around −6.5‰, typical of carbon in most of carbonatites[7,8], diamonds[9] and kimberlites[10], have been found in gases from northern Afar[4] (P.A. and M. Javoy, in preparation). The high-temperature gas samples collected from the November 1978 Asal rift eruption, southern Afar[1], have provided new isotope data on basaltic igneous volatiles, released from upper mantle magmatism[11].

Four gas samples were collected from the fissure which generated the eruption ($T = 1{,}070$ °C) and another four samples were taken from outpoured lava flows, 60 m away from the fissure ($T = 800$ °C)[1].

Chemical compositions were determined by gas chromatography and are reported in Table 1 together with the isotopic results. For isotopic measurements, CO_2 and SO_2 were separated from the mixtures by differential freezing in a high vacuum Pyrex line: after the non-condensible gases in liquid nitrogen were pumped (CO, H_2, COS, N_2, O_2 and Ar), melting dimethylbutane (−140 °C) and dry ice acetone (−80 °C) traps were used to isolate CO_2 from SO_2, and SO_2 from residual water vapour, respectively. Complete extraction was controlled with a precision pressure gauge, and the proportions of gas recovered agreed with the chromatographic results (±2%). Isotopic analyses were performed on a double-collecting micromass spectrometer. Results are expressed in the usual δ notation (1) (ref. 12). Standards are PDB for carbon, SMOW for oxygen and Canyon Diablo Troïlite for sulphur. The accuracy of the mass spectrometric analyses (1σ) is better than 0.1‰ for carbon and oxygen, 0.3‰ for sulphur.

$$\delta = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 1{,}000, \qquad (1)$$

where $R = {}^{13}C/{}^{12}C$ or $^{34}S/^{32}S$.

Although the samples are largely air contaminated, as shown by their high N_2 contents and $O_2 + Ar/N_2$ ratios which are similar to the atmospheric ratio (0.280), the corresponding CO_2 air contribution amounts to <1% of the total CO_2 content of the samples, and is only significant in the 800 °C samples, particularly in sample no. 13. Assuming that ~0.03 mol% of atmospheric CO_2 with a $\delta^{13}C \simeq -7$‰ were added to those samples which contain about 78 mol% of N_2, we can restore the $\delta^{13}C$ of true volcanic CO_2: corrected values are in parentheses in Table

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Table 1 Chemical and isotopic compositions of gases from the Asal eruption

	Erupting fissure (1 or 2) (1,070 °C)				Lava flows (800 °C)			
Sample:	1 A92	1 A89	2 A88	2 G82	no. 6	no. 13	no. 5	no. F3
H_2O (mol%)	79±3	79±7	ND	77±7	ND	ND	ND	ND
Dry volcanic gases (mol%)	21	21	—	23	—	—	—	—
CO_2	18.9	18.7	15.9	14.2	90.7	99.9	94.4	93.2
SO_2 (mol%	80.4	80.8	84.0	84.9	9.3	0.1	5.6	6.8
H_2S of dry	0.0004	0.02	0	0.01	0	0	0	0
H_2 volcanic	0.25	0.06	0.05	0.22	0	0	0	0
CO gases)	0.35	0.30	0.22	0.59	0	0	0	0
COS			2×10^{-4}–10^{-3}					
C/S	0.24	0.23	0.19	0.18	9.7	10^3	17	14
N_2 (mol% of dry gases in samples)	45.0	50.9	47.4	57.9	81.0	78.6	80.3	80.4
O_2+Ar/N_2	0.244	0.251	0.236	0.266	0.180	0.273	0.178	0.188
$\delta^{13}C\pm0.1‰$ vs PDB(CO_2)	−5.9	−5.3	+0.2	−4.8	+2.3 (+2.4)	+1.5 (+2.2)	+1.9 (+2.0)	+2.1 (+2.2)
$\delta^{18}O\pm0.1‰$ vs SMOW(CO_2)	+8.2	+7.8	+8.6	+8.2	+16.0	+20.8	+16.1	+15.9
$\delta^{34}S\pm0.3‰$ vs CD(SO_2)	−3.1	−2.9	−2.3	−2.8	−0.1	ND	−4.8	−0.2

ND, not determined; 0 means under the limits of detection. All the samples were collected in previously evacuated Pyrex bottles containing P_2O_5 desiccator in excess to trap water vapour. A silica collection pipe was used to lead the gases into the bottles[1].

1. Except for sample 13, corrections are within the accuracy possible for mass spectrometric analyses and allow a reliable determination of the $^{13}C/^{12}C$ ratio in volcanic CO_2.

CO_2 accounts for nearly the whole carbon content of the gases, as CO and COS when detected are only minor components. Note that both reverse C/S atomic ratios and opposite-sign $\delta^{13}C$ values distinguish the 800 °C samples (C/S > 10, $\delta^{13}C>0$) from 1,070 °C samples (C/S = 0.2, $\delta^{13}C<0$). Such a discrepancy, and such a high $\delta^{13}C$(+2.2‰ versus PDB) in the gases from lava flows, were surprising: the exhibited value fits in with the isotopic range for ^{13}C-rich marine limestones[6].

This might be related to the presence, near to the eruption point, of lacustrine carbonates deposited from the Asal lake basin (155 m below sea level) since this one was isolated from the marine gulf of Ghoubbet–Tadjurah and extended over a wider area of the Asal graben's floor than it is at present[13]. Carbonates were collected in the field near to the sampling sites for chemical and isotopic composition analysis (Table 2).

The results ($\delta^{13}C=+0.85‰$) show that a thermal decomposition of such a magnesium-rich calcite could produce CO_2 with a $\delta^{13}C$ similar to that found in the 800 °C samples: if we assume that isotope equilibrium was maintained between the carbonate and the CO_2 released, and that the fractionation factor for calcite can be used, then a 1.4‰ ^{13}C enrichment in CO_2 would correspond to an equilibrium temperature of about 800 °C (ref. 14). Isotopic equilibrium conditions may have been maintained by the thickness of the lava field (10m) that allowed the gases to escape only from a few points where the upper lava crust was broken. Thus, carbonate sediments, partly overlapped by the lava flows, seem the most probable source for the CO_2 sampled outside the fissure.

$\delta^{13}C$ of sample A88 (+0.2‰), collected from one of the two vents we used above the erupting fissure, suggests a local mixing between a light magmatic CO_2 and the carbonate-derived CO_2. If we consider the $\delta^{13}C$ value from sample A92 ($\simeq-6‰$) the most representative of unmixed carbon dioxide, we may evaluate the possible contributions from carbonate-derived CO_2 to the other three samples: 9%, 76% and 15% for samples A89, A88 and G89, respectively. It is possible that superficial CO_2 may also have made a (small) contribution to sample A92.

This −6‰ value for uncontaminated CO_2 from the fissure is very similar to that of magmatic CO_2 from the Erta Ale lava lake, on the northernmost spreading axis in Afar ($-6.4\pm0.4‰$)[4]. It could also be compared with the $\delta^{13}C$ of CO_2 trapped in fresh tholeïtic basalts from the mid-Atlantic ($-7.6\pm0.5‰$)[15] and east Pacific (−5.4 to −5.8‰)[16] sub-oceanic ridges. Then, it falls within the isotopic range for most of carbonatites, diamonds and kimberlites in which carbon is usually considered to derive from a deep-seated source.

The shallowness of the mantle beneath the Asal–Ghoubbet ridge[11], the thinning of the oceanic crust beneath the axis indicated by the seismic profiles[17], the lack of continental-type crust, the axial situation of the eruption point[1], the high temperature of the gases and their close relationship to the magma feeding channel, suggested that the mantle would be a probable source of the CO_2 released from the fissure. The consistency of the $\delta^{13}C$ obtained with that of most of presumed deep-seated carbon-rich reservoirs supports such an origin. The close similarity of the $^{13}C/^{12}C$ ratios in Asal and Erta Ale magmatic gases indicates that the mantle beneath Afar's spreading axis would have a uniform carbon isotope composition, comparable with that of carbon from other sub-oceanic ridges[15–16].

The much lower values measured in magmatic gases from Hawaii[2,3,5] suggest either (1) lighter $^{13}C/^{12}C$ ratios in Hawaiian magma sources, the carbon in plumes from deep mantle being relatively ^{13}C-depleted compared with carbon in shallow mantle rises beneath oceanic ridges; or (2) only local contributions as some of the gases were collected from lavas having flooded over organic material[2]. The difference between Asal samples from the fissure and outside the fissure emphasises that lava flows are inadequate sampling sites, being easily contaminated by environmental fluids.

SO_2 constitutes the whole sulphur content of the gases, as H_2S and COS are trace components. Thermodynamical equilibrium in collection conditions (1 atm, 1,070 °C and $p_{O_2}=10^{-9.4}$ atm)[1] would lead to higher H_2S/SO_2 ratios than are found in the samples (8×10^{-3} compared with 10^{-4}). The lower O_2+Ar/N_2 ratios in samples than in air indicate that high-temperature air oxidation would be responsible in the reduction of the H_2S proportions. Residual traces of H_2S could also have later reacted with SO_2 in the sampling bottles, according to the reaction

$$2H_2S+SO_2\rightleftarrows3S+2H_2O$$

which brings about a relative ^{34}S enrichment in SO_2. But the lack of sulphur precipitation in the bottles during the few days that they were waiting for analysis suggests that small proportions of H_2S compared with SO_2 made this reaction inefficient. (Water vapour, which favours the reaction, was trapped on P_2O_5 desiccator.) Hence, the $\delta^{34}S$ of SO_2 would account for the original $\delta^{34}S$ of total sulphur in the gases. $\delta^{34}S$ in 1,070 °C samples are homogeneous, around a mean value of −2.8‰ versus CD standard; those in 800 °C samples are less homogeneous, but they enclose the former value.

Table 2 Chemical and isotopic compositions of lacustrine carbonates from Asal rift

	(wt%)	$\delta^{13}C$ vs PDB (‰ ± 0.1)	$\delta^{18}O$ vs SMOW (‰ ± 0.1)
$CaCO_3$	55%		
$MgCO_3$	28%	+0.82	+44.2
Amorphous silica	17%	+0.88	+44.6

Chemical analyses were carried out using an X-ray energy dispersion spectrometer. For isotopic analyses, 1 mg of carbonate was reacted with 100% H_3PO_4 acid at 50 °C for 12 h, after heating 390 °C for 45 min.

This −2.8‰ $\delta^{34}S$ is low in the classical range for igneous sulphur in basaltic rocks[18–20] and fumarole gases[21], the average composition of which usually lies close to the meteoritic standard value. Comparison with the $\delta^{34}S$ of SO_2 from Erta Ale, +1.0 ± 0.3‰ (P.A. and M. Javoy, unpublished data), suggests that sulphur in Asal gases originated from a slightly ^{34}S-depleted source, unless continuous loss of SO_2 involved a progressive ^{34}S depletion in the residual sulphur which escaped from the magma, as the feeding intrusion seems to have been a rather closed system[1] and our samples were collected during the later degassing trend of the eruption. If we assume that the initial $\delta^{34}S$ of total sulphur in the magma was near zero, that about 90% of the original sulphur content had already escaped at the collection time and that a Rayleigh-type isotope distillation took place between SO_2 and the magmatic sulphides, then a fractionation factor of 1.00122 is obtained; this is compatible with the value extrapolated from Sakai's data for the SO_2/S^{2-} equilibrium fractionation at temperatures of about 1,200 °C (ref. 22). Otherwise, kinetic isotope fractionation would have concentrated ^{34}S in the residual sulphur and led to a higher ratio than is observed.

This relatively low $\delta^{34}S$ excludes superficial contributions to the volcanic fluids from sulphates in the marine waters which circulate into the graben from the Ghoubbet to Asal lake (3,900 mg l^{-1})[23] and in the saline waters of the lake itself (4,300 mg l^{-1}) which is 2 km away from the eruption point: the SO_2 produced by high-temperature reduction of such sulphates ($\delta^{34}S \simeq +20$‰)[23] would actually be ^{34}S-rich compared with SO_2 in the gases, isotope fractionation between SO_4^{2-} and SO_2 remaining close to zero at temperatures above 600 °C (ref. 22). A similar lack of chloride contributed by the environment was previously reported from chemical analyses of gases and related sublimates[1].

Thus, carbon and sulphur outgassed from the fracture which generated the recent Asal eruption derived from a probable mantle source because of both the exceptional tectonic setting of the Asal rift and their stable isotope composition which was similar to that of comparable deep-seated material.

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The Lizard complex as an ophiolite

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The Lizard complex of south Cornwall consists of an assemblage of peridotites, gabbros, hornblende schists, metasediments, dolerites and metadolerites and acid-basic banded gneisses (the Kennack gneisses). It is bordered to the north by a zone of chaotic sediments with phacoidal inclusions of pillow lavas, greywackes and quartzites (the so-called Meneage crush zone). This zone can be traced eastwards into the Roseland district of south Cornwall[1]. The age of the complex is uncertain. Dates (largely K–Ar) obtained from the Kennack Gneiss and the majority of hornblende schists are mainly between 370 and 390 Myr, although Green[2,3] considered the peridotite to be older than the oldest date obtained from the hornblende schists, at 492 Myr. Metadolerite dykes have recently[4] been given ages of approximately 400 Myr. All the above are thought to represent minimum ages due to the possibility of argon loss during subsequent uplift, cooling and mild metamorphism. Since the work of Green[3], the Lizard complex has been regarded by many as the type example of a hot diapiric mantle intrusion into continental crust. Intrusion is thought to have occurred during a period of regional metamorphism and to have resulted in the imposition of a contact dynamothermal aureole on the hornblende schists at the margin of the peridotite body. The gabbro is regarded as a later and unrelated intrusion, and the Kennack gneisses were attributed to intrusion of acid magma along sheared basic dykes in peridotite. Thayer[5] suggested that the complex was ophiolitic, basing his views on the field relationships of gabbros and peridotites which indicated that the two lithologies might be temporally and genetically related. General comparisons have since been made between the Lizard complex and the well-documented ophiolote sequences[6–10], although little detail has generally been given. I provide here relevant details which suggest that the Lizard complex is ophiolitic in origin.

The east coast section of the Lizard complex is thought to form a rock association distinct from the remainder of the complex and to be separated by a major fault (Fig. 1*a*). There are four east–west trending divisions of the east coast section, which from north to south are: a dyke complex in gabbro; massive, variable and layered gabbro; troctolites and related rocks in peridotite; and peridotite.

The dyke complex consists of subequal amounts of variable gabbros and NW–SE trending subvertical tholeiitic metadolerite dykes. Small amounts of hornblende leucodiorite form segregations in the gabbro and have locally intruded fractured metadolerite dykes forming net-veined complexes. The base of the dyke complex is at Godrevy Cove where there is a rapid transition southwards into gabbro containing only a small percentage of dykes. Subvertical, E–W trending cumulate layering produced by varying proportions of olivine, plagioclase and occasionally clinopyroxene is locally preserved; rare mineralogically graded units 'young' to the north. The present orientation of the cumulates demonstrates that the whole sequence has been rotated through a large angle downwards to the north about an east–west axis. These cumulates are thought to have been deposited on a subhorizontal or gently dipping surface on a floor of peridotite, although subsequent gabbro intrusion and high temperature deformation have obscured many of the original relationships. The remaining gabbro has less well-defined mineralogical variations and is variable between strongly laminated (mostly the plagioclase-rich varieties) and non-laminated. The contact with peridotite at

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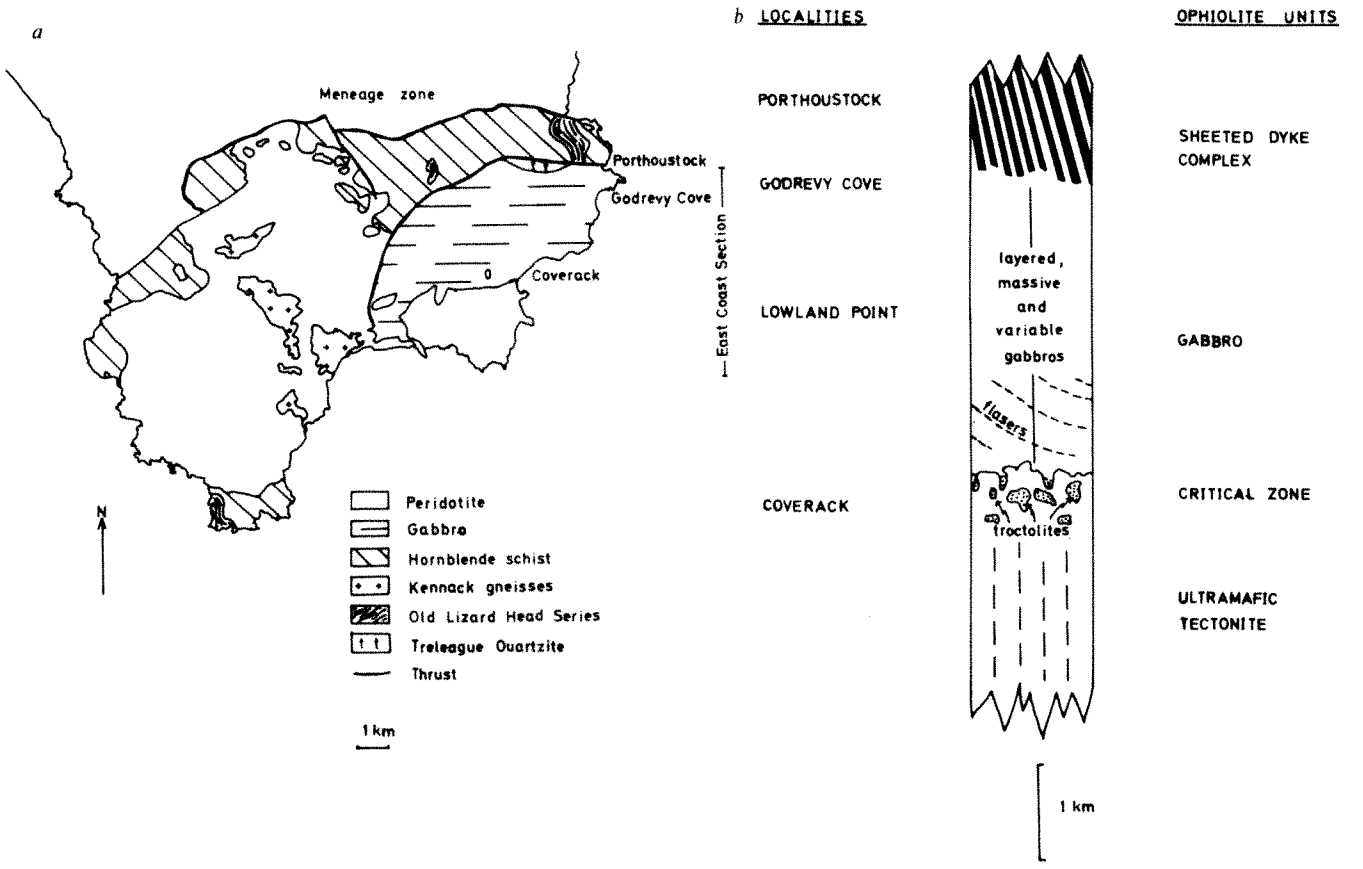


Fig. 1 *a*, Simplified geological map of the Lizard complex. *b*, Schematic section of the east coast section and a comparison with an ophiolite succession.

Coverack in the south is a broad zone in which olivine-rich gabbroic and ultramafic rocks ('troctolites') ranging from dunites to olivine gabbros, are complexly interdigitated with peridotite. The troctolites are considered to be the first (and therefore olivine-rich) cumulates which separated from the gabbroic magma, which overlay the peridotite and which were subsequently deformed and partially remobilised, resulting in the inclusion of blocks of peridotite. A possibly slow rate of removal of melt from the peridotite may have resulted in the precipitation of troctolites in pods within the peridotite. Partial-melt textures in the host peridotite are encountered locally in this zone. Rudely-banded lherzolites and harzburgites containing little or no troctolite outcrop further to the south.

The similarity in both the lithologies present and the order in which they occur to the rock succession encountered in an ophiolite sequence (Fig. 1*b*) is striking and considered to be sufficient evidence to accept this as a truncated ophiolite sequence. This interpretation is in complete contrast to that of Green[3] who considered the gabbro to form part of an *in situ* ring intrusion into peridotite.

Of prime importance to the proposed ophiolitic nature of the complex is the relationship between peridotite and gabbro; in ophiolites these are thought to have a parent–daughter relationship. Gabbro in the Lizard complex occurs only within peridotite and not in other lithologies—fortuitous if these two lighologies are not related. Examination of Green's lithological map and the author's field observations show that the gabbro is further restricted to the primary-type peridotite and does not occur in the recrystallised varieties except as isolated and deformed scraps. This implies that the association of peridotite and gabbro existed before their juxtapositioning with the surrounding hornblende schists (the event in which some of the peridotite was recrystallised), a feature common to many other ophiolites. Partial-melt textures at Coverack and locally elsewhere involve gabbroic veins with gradational boundaries against the surrounding peridotite. These support a genetic relationship, which is supported further by the relative rare earth contents of the two lithologies[11]. Thus the proposal for the ophiolitic nature of the complex is strengthened.

The remainder of the Lizard complex, which contains very little gabbro is considered to be the peridotite component of the ophiolite sequence together with the underlying metabasic and metasedimentary sub-ophiolitic rocks (the hornblende schists, the Old Lizard Head series rocks and the Kennack gneisses). These lithologies will be reported elsewhere but the salient points are noted here. Two varieties of hornblende schist were distinguished by Flett[12] and subsequently by Green[3]—the Traboe and the Landewednack hornblende schists. The Traboe variety occurs near the peridotite and was considered to be the upper amphibolite and granulite facies derivative of the amphibolite-grade Landewednack variety, the agent of metamorphism being the intrusive peridotite. Chemical data, however[13], show that the two groups are chemically distinct and cannot therefore be metamorphic equivalents. The Traboe schists are chemically more 'primitive' than the Landewednack schists. Both the chemical and field evidence support derivation of the Traboe schists from dominantly intrusive and cumulate gabbroic rocks and the Landewednack schists from dominantly intrusive and extrusive basaltic rocks. All are tholeiitic and those which were derived from basaltic protoliths have an oceanic chemistry (Fig. 2). It is proposed that the hornblende schists were derived by deformation of an ophiolite sequence adjacent to and chemically slightly different from that of the east coast section. The transition from the Landewednack variety to the Traboe variety reflects the original transition from the extrusive basaltic to the cumulate gabbroic portion of the ophiolite. True prograde metamorphic sequences are thought to be restricted to those schists below the peridotite from Kennack southwards to Landewednack. Much of Green's prograde metamorphic sequence is thought to be a feature of retrogression.

The Kennack gneisses show abundant evidence of formation by an *in situ* segregation process, in contrast to the arteritic origin proposed by Green, although this is undoubtedly locally true. Detailed petrographic evidence together with the

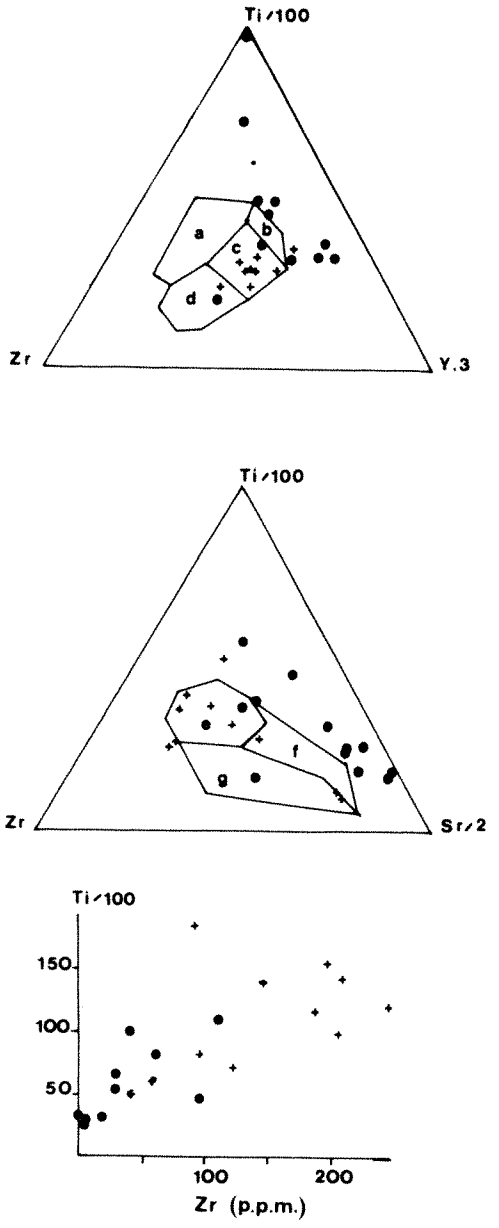


Fig. 2 Geochemistry of the hornblende schists. +, Landewednack; ●, Traboe. Field divisions from Pearce and Cann[28]: a, within place basalts; b, c, low potassium tholeiites; c, ocean floor basalts; c, d, calc alkaline basalts; e, ocean floor basalts; f, island arc basalts; g, Calc alkaline basalts. The large scatter of data points of the Traboe is a direct result of their low incompatible element contents resulting from their probable derivation from cumulative gabbros.

chemistry of the acid and basic portions suggest that the most likely process was one of partial anatexis of sediments and volcanics. The spatial restriction of the gneisses and the parallelism of the banding to the margin (base) of the peridotite suggests that this was achieved during overriding of the hot peridotite body. The deformation during assembly of the Lizard complex is thought to have resulted in the recrystallisation of the margins of the peridotite to lower pressure assemblages (the recrystallised anhydrous and hydrous varieties of Green[3]).

The question remains, however, as to whether this ophiolite is a fragment of oceanic crust, as is commonly proposed for many other ophiolites[14–17]. The complete absence of rocks of continental affinities within the east coast section is thought to be strong evidence in support of an oceanic origin for the complex. Deformation of the gabbroic cumulates occurred throughout their cooling history and ranged from slumping of unconsolidated cumulates through crystal-mush deformation in a direction subparallel to the layering to the formation of flaser gabbros and finally to brittle faulting. Flaser gabbro zones are concentrated in the south of the gabbro in the lower part of the original cumulate pile and represent low-angled slides developed under amphibolite-facies conditions during cooling of the gabbro. The above, combined with the large degree of crustal extension indicated by the dykes of the dyke complex, demonstrate that crystallisation of the complex occurred in a dominantly tensional and tectonically unstable environment. This is certainly consistent with an origin of the complex at a constructive plate margin. Chemical analyses of the basaltic dykes (ref. 13 and G.A.K., in preparation) indicate a close similarity in both major and trace-element compositions to oceanic tholeiites (Fig. 3). The gabbroic cumulates have tholeitic affinities and are also thought to have been derived from an oceanic tholeiite magma. Geochemical evidence therefore also supports an interpretation of the Lizard ophiolite as a fragment of oceanic crust. If present models for the generation of oceanic crust are correct[18–20], then the gabbroic cumulates and dykes should be derived from the same primary magma. The geochemistry of the dykes at Porthoustock and the gabbroic cumulates of the Lizard broadly support this proposal (Fig. 4), although these results are at variance with those of Floyd *et al.*[21].

The extrusive portion of the ophiolite sequence is absent south of the Lizard boundary thrust. However, pillow lavas occur within the Meneage crush zone, which is believed to be a

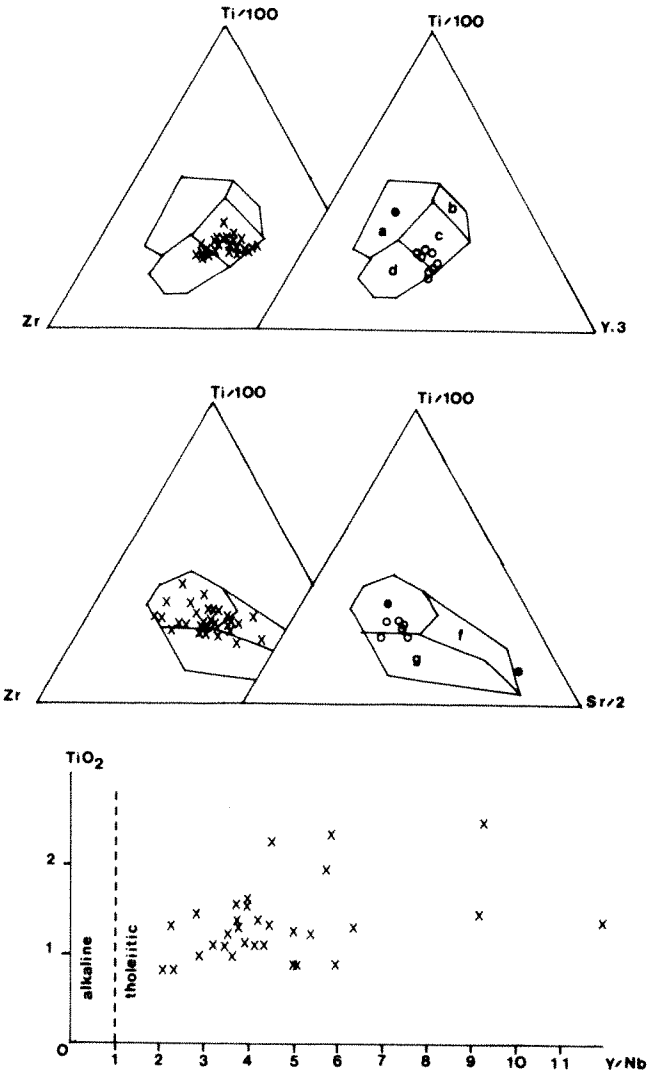


Fig. 3 Geochemistry of basaltic dykes (×) and lavas (○). ●, Average SW England tholeiite. Fields as for Fig. 2. Alkaline–tholeiite discriminant diagram from Floyd and Winchester[29].

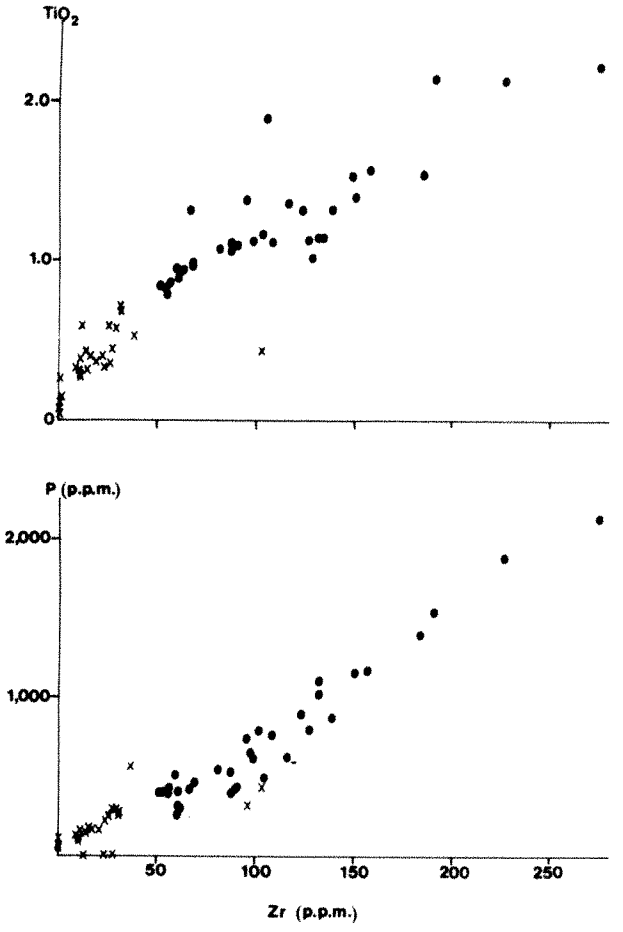


Fig. 4 Interrelationship of trace-element contents of dykes (●) and cumulate gabbros (×). Approximately straight-line trend with gabbros plotting at low concentrations supports a cogenetic relationship.

melange produced beneath and in front of the Lizard ophiolite during its emplacement. The chemistry of the contained lavas is closely allied to rocks of the Lizard complex (Fig. 3) and quite different from the remaining greenstones of South-west England[1,13,22]. It is therefore thought possible that these lavas are part of the extrusive portion of the Lizard ophiolite or the surrounding oceanic crust which was incorporated into an olistostrome ahead of the ophiolite during its emplacement, and which were subsequently overridden.

The acceptance of the Lizard complex as obducted oceanic crust has important implications for the geological history of SW England. Thus models such as that of Floyd[23] in which SW England was never underlain by oceanic crust are thought to be, in part at least, incorrect. However, I would support Floyd's interpretation of the geochemistry of other SW England greenstones in suggesting that the ocean crust did not extend anywhere north of the Lizard boundary thrust or its extension eastwards. Equally, therefore, the models of Johnson[24], Burret[25], Laurent[26] and Burne[27] are thought to be incorrect in suggesting that all of SW England was underlain by oceanic crust. A knowledge of the age of generation and emplacement of this oceanic crust is important in the development of geotectonic models for this region. It is thought that the ages of up to 403 Myr (late Silurian) obtained from the dykes of the dyke complex[4] most closely approximate the age of generation. Dates obtained from the gabbro would also be definitive (work on this is in progress). The concentration of ages determined from the hornblende schists and the Kennack gneisses (370–390 Myr)[2] are thought to date the assembly and beginning of emplacement of the ophiolite. The short time between the generation and emplacement of the complex is a feature common to many ophiolites.

In conclusion it is thought that many features of the Lizard complex do not support an origin by diapiric intrusion of peridotite into continental crust. The geological and geochemical features are consistent with formation as part of late Silurian (?) oceanic crust followed by emplacement of the resulting ophiolite sequence over the continental margin of SW England in early Devonian times.

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High sediment yields from major rivers of the Western Southern Alps, New Zealand

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Suspended sediment yields have been determined by combining suspended sediment rating relationships with water flow records for gauged rivers of South Island, New Zealand. Rivers draining the Western Southern Alps have specific annual yields about 10 times higher than world average rates for mountainous areas. Their short steep catchments, rising in elevation from near sea level to over 3,000 m, support dense unmodified podocarp hardwood and beech forests below 1,000 m. The drainage basins are virtually undisturbed. A simple positive power law relationship between specific annual suspended sediment yields and the extremely high mean annual rainfalls explains almost all the variance in yield for catchments of the western region. This simple law may also hold for undisturbed catchments having a temperate maritime climate, in other areas of the world.

Suspended sediment yields (Table 1) were calculated by integrating suspended sediment rating relationships with continuous flow records of the rivers. These rating relationships are of the form

$$C = aQ^b$$

where C is the suspended sediment concentration in kg m^{-3} and Q is the water discharge in m^3 s^{-1}. Exponents are similar for all major rivers ($b = 2.2$ to 2.7); coefficients vary through several orders of magnitude ($a = 1.80 \times 10^{-3}$ to 1.87×10^{-5}) partly reflecting the wide variation in catchment areas. The range of suspended sediment concentration values, displayed in Fig. 2 for two typical rivers, is not exceptional. Stage discharge ratings on the unstable channels are defined by a regular gauging

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Table 1 Mean annual specific suspended sediment yields and other relevant data for gauged East Coast major rivers and for all gauged West Coast rivers of South Island, New Zealand

	Catchment area (above gauging station) (km^2)	Mean annual specific suspended sediment yield (tonnes km^{-2} yr^{-1})	Mean annual rainfall (mm)	Mean annual flow (m^3 s^{-1})	Period of sediment record (yr)
East Coast					
Wairau	3,430	870	1,600	114	17
Waiau	1,980	1,300	2,000	90	10
Waimakariri	3,210	1,670	1,900	120	5
Rakaia	2,640	1,640	3,000	200	20
West Coast					
Karamea	1,160	330	3,300	105	10
Buller A	4,560	270	2,400	240	15
Buller B	6,350	270	2,600	416	14
Inangahua* A	234	170	2,500	13	13
Inangahua* B	1,000	730	3,000	73	14
Grey	642	550	3,000	48	8
Butchers*	4	270	2,900	0.30	7
Hokitika	352	17,070	9,400	99	7
Haast	1,020	12,730	6,500	193	9
Cleddau	155	13,300	7,000	32	15

* Minor river.

programme. Unlike water flow gaugings, concentration measurements have mostly been made during floods having peak discharges less than the mean annual flood but ~75% of the sediment is transported by these lower peak discharge, higher frequency events[1].

The algorithm used for the computer calculation of yield was

$$G = (1/1000AT) \sum_{i=1}^{n} 0.25(C_i + C_{i+1})(Q_i + Q_{i+1})\,\Delta t$$

in which A is the catchment area in km^2, T is the duration of the water flow record in years, n is the number of observations, i is a summation integer and G is the specific suspended sediment yield in tonnes km^{-2} yr^{-1}. At a gauging station the water level is recorded every 900 s and thus Q at the same interval may be derived from a flow rating. It was found to be sufficiently accurate, however, to use a value of 3,600 s for Δt in the above algorithm. It has been suggested[2] that the absolute errors associated with calculation of sediment yield by methods similar to the above, could be as high as +60% for annual yields. Unfortunately, absolute errors for the data of Table 1 cannot be assessed owing to the absence of measurements made by an alternative precise procedure. The regional consistency of yields demonstrated in Fig. 3 does suggest, however, that the relative error between rivers is small.

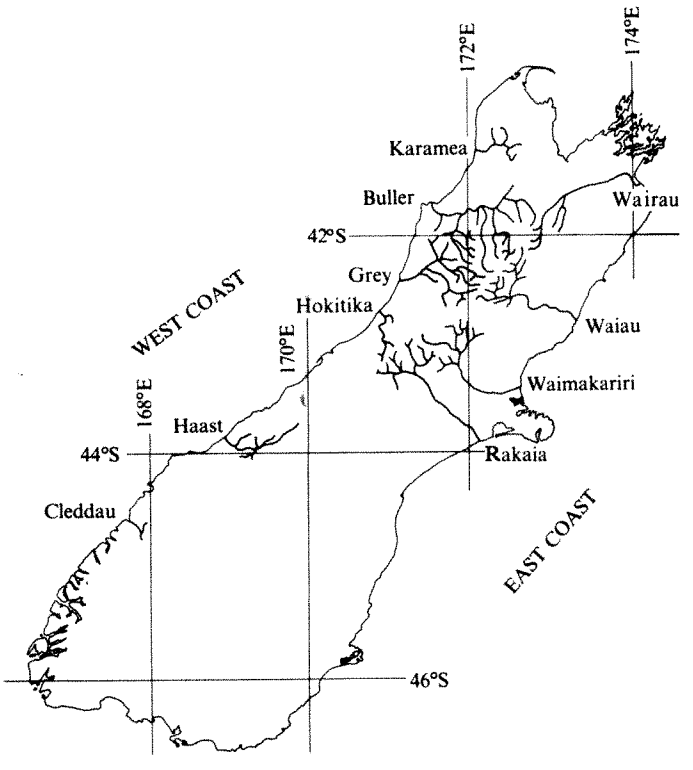


Fig. 1 Map of South Island, New Zealand, showing the location of major rivers gauged for suspended sediment. Other rivers were either not measured or have sediment storage basins (lakes) upstream of gauging stations.

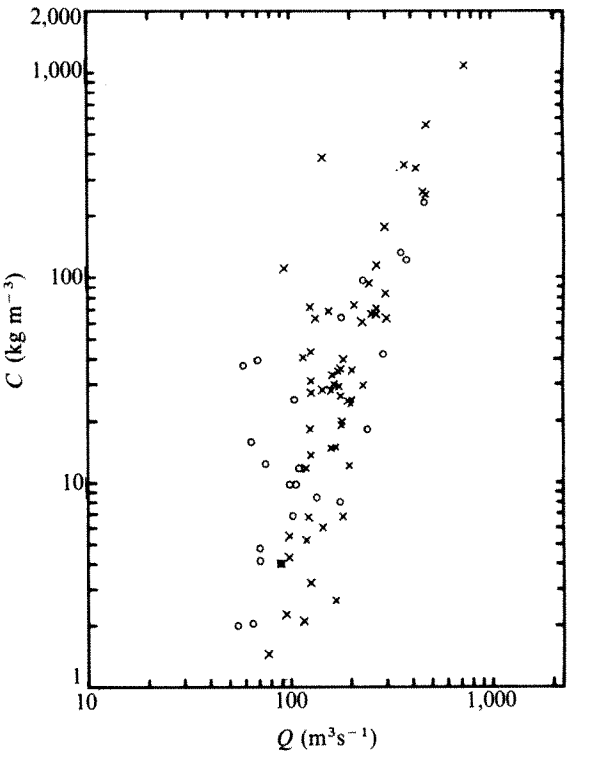


Fig. 2 Suspended sediment concentration, C plotted against water discharge, Q, for the Rakaia River (×) and the Haast River (○).

Catchments of very different geology, for example, that of the Rakaia River on well indurated sandstone and that of the Haast River on fissile schist, have nearly identical suspended sediment rating relationships (Fig. 2). Furthermore, West Coast rivers (Fig. 1) are steeper, have coarser gravel beds and shorter braided lengths than others in South Island, as they occupy gorges for most of their extent.

Specific yields calculated for East Coast rivers (Table 1) are in general agreement with earlier crude estimates (S. Thompson and J. Adams, personal communication). The world average denudation rate for mountainous terrain is estimated[3] to be 1,300 tonnes km^{-2} yr^{-1}. Rates for East Coast catchments lie close to this figure. (Bedload and dissolved load is not included in Table 1, but the few measurements available suggest that these components rarely exceed 10% of the total load[1].)

There is a spectacular rise in specific water and sediment yields and in mean annual rainfall south of the Grey River. The specific sediment yield of the Hokitika River is enormous: it is 2.4 times higher than that of the Ching River in China, quoted[4] as having the largest measured specific yield of major rivers of the world.

A linear multiple regression analysis between sediment yield as the dependent variable and independent variables, including catchment area, mean annual rainfall, mean annual discharge, mean annual flood, 10 yr recurrence interval flood, mean annual runoff, mean annual flood runoff, 10 yr recurrence interval flood runoff, mean basin elevation above sea level, river channel

length, mean catchment slope and percentage forest cover—for West Coast catchments (Table 1) indicated that 95% of the variance in suspended sediment yield for the region is accounted for by mean annual rainfall (Fig. 3). All variables used in the analysis were logarithmically transformed. Geology varies widely from granite to indurated sandstone and mudstone to fissile schist on West Coast and, as implied earlier, variations are apparently of little significance in suspended sediment production.

Current analysis may, however, reveal the relative influence of further parameters.

The simple positive power law relationship between suspended sediment yield and rainfall, P, defined in Fig. 3 by

$$G = 9.02 \times 10^{-11} P^{3.65}$$

confirms previous work which has pointed to this positive trend[5-10].

In terms of mean annual runoff, R (mm) the relationship is

$$G = 3.51 \times 10^{-8} R^{3.00}$$

which explains 93% of the variance in yield and reflects, in part, the poor water storage capacities of the catchments involved.

The yield/rainfall relationship of Fig. 3 may be typical of other very high rainfall areas such as parts of the Himalayas[11,12], New Guinea[13,14], Japan[15], Java[16] and Taiwan[17] where large specific yields have been reported. Taiwan is of particular interest, for within the hardwood forest covered, steep Central Range the average specific suspended sediment yield is 13,000 tonnes km^{-2} yr^{-1} which is similar to the average value (14,000 tonnes km^{-2} yr^{-1}) for rivers south of the Hokitika River, South Island. Moreover, gross rainfall values[18] exceed 5,000 mm for the higher yielding Taiwan catchments and are comparable to the values of 6,000–7,000 mm for catchments south of the Hokitika River.

The highest specific yield in Taiwan, measured on a minor river[17], is 31,700 tonnes km^{-2} yr^{-1} perhaps the highest known value in the world. But if the relationship in Fig. 3 is valid regionally, then in minor south West Coast drainage basins, where mean annual rainfalls surpass 10,000 mm, yields above 36,000 tonnes km^{-2} yr^{-1} probably exist.

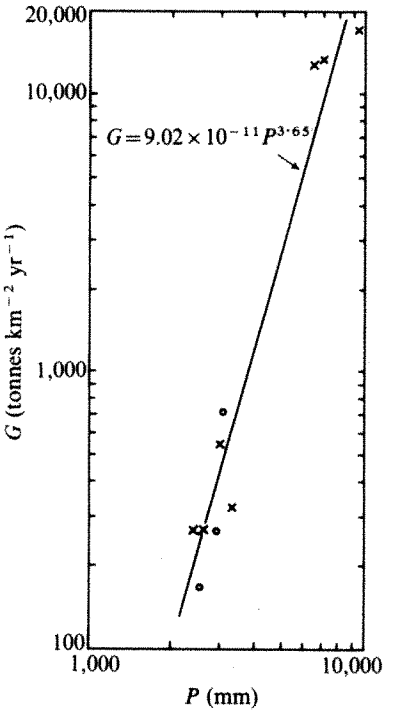


Fig. 3 Mean annual specific suspended sediment yield, G, plotted against catchment mean annual rainfall, P, for major rivers (×) and minor rivers (○) of the Western Southern Alps, New Zealand.

Predictions of suspended sediment yield as a function of precipitation in semi-arid or arid[5,9], subhumid[7,10], and tropical[5,7], conditions have been given elsewhere. None of these relationships apply to the West Coast region where temperate, maritime conditions exist. In such a climate, for undisturbed catchments, a simple power law expression between yield and rainfall may provide useful estimates of specific suspended sediment yields.

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Response of deep-sea benthonic Foraminifera to development of the psychrosphere near the Eocene/Oligocene boundary

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A dominant feature of present-day thermohaline circulation in oceans is the production of cold Antarctic Bottom Water (AABW) in the Weddell and Ross Seas and along the continental margin off Antarctica. The cold, dense AABW formed at or near the surface sinks to abyssal depths and travels throughout the ocean basins. AABW circulation was suggested to have begun near the Eocene/Oligocene boundary with the development of the psychrosphere (lower cold layer of a two-layer ocean with temperatures <10 °C) inferred from ostracode faunal data[1] and oxygen isotopic evidence from closely-spaced samples in DSDP site 277 (ref. 2). Previous studies suggested that the temperature change associated with the development of the psychrosphere caused a crisis for deep-sea ostrocodes[1] and deep-sea benthonic Foraminifera[3]. I consider here the response of benthonic Foraminifera to the development of the psychrosphere.

Benthonic Foraminifera in 15 closely-spaced sediment samples from DSDP site 277 have been examined. Comparison of the faunal data and the oxygen isotopic record reveals that the temperature drop coincides with a pronounced reduction of the frequency of *Epistominella umbonifera*. Few other faunal changes (first or last occurrences) are recorded at the time of the development of the psychrosphere, although 16 faunal changes occurred before and six occurred after the isotopic event. Taxa which exhibit first and last occurrences are all rare forms. I suggest that the psychrosphere may have developed gradually during the middle–late Eocene, culminating in a rapid temperature drop near the Eocene//Oligocene boundary.

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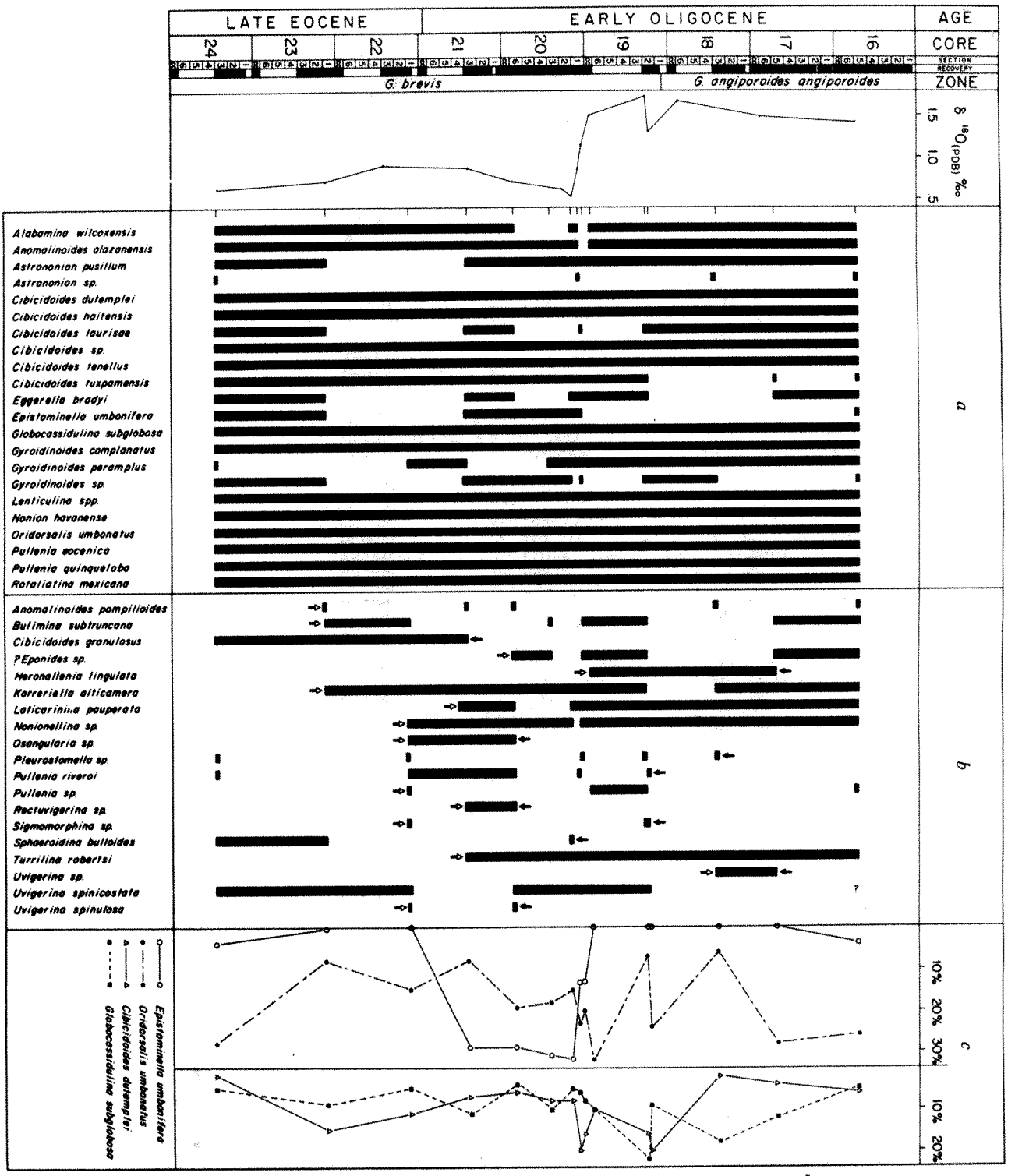


Fig. 1 Summary of benthonic foraminiferal biostratigraphic data from DSDP site 277. The inferred age[2], core recovery, planktonic foraminiferal zones[9], and isotopic curve based on mixed benthonic foraminifera[2] are shown left, with the tick marks indicating the samples examined for the benthonic foraminiferal analysis. Biostratigraphic ranges of 41 taxa are shown: *a*, taxa that lack first or last occurrences in the sequence; *b*, taxa that exhibit first or last occurrences. First appearances are marked by open arrows, and last occurrences are marked by solid arrows. *c*, Quantitative data of four dominant benthonic foraminiferal species.

DSDP site 277 is located on the Campbell Plateau (52°13.43′ S, 160°11.48′ E, 1,222 m) in sub-Antarctic waters south of New Zealand in the Southern Ocean. The sequence in site 277 consists of continuously cored foraminiferal-rich calcareous sediments[4]. The Campbell Plateau at 38 Myr was at about palaeolatitude 65 °S (ref. 5).

The oxygen isotope curve based on mixed benthonic Foraminifera for the late Eocene–early Oligocene (Fig. 1) is assumed to reflect temperature variation of the bottom waters[2], as the Antarctic continent is considered to have been essentially ice-free during the Eocene and Oligocene[6]. A reduction in bottom-water temperatures of about 5 °C was recorded in the earliest Oligocene. It has been suggested that the development of the psychrosphere resulted from this temperature change, reflecting a decrease in Antarctic surface water temperature to freezing, the production of the first extensive sea ice and the onset of cold bottom-water production[2]. The initiation of vigorous bottom-water circulation is thought to have caused widespread erosion of sediments, increased calcium carbonate dissolution in some areas, and caused a precipitous drop in the calcium carbonate compensation depth in other areas[2].

Oxygen isotope analysis of monospecific samples of the benthonic foraminifer *Oridorsalis umbonatus* in this section was recently carried out, and the temperature drop seems to be about 4 °C, (L. Keigwin, personal communication) rather than 4–5 °C as previously thought[2], but this drop is still significant. The exact stratigraphic position of core 19 samples relative to core 20 is unknown because of incomplete core recovery, and the rate of temperature decrease cannot be precisely determined, although a rapid reduction within 100,000 yr has been suggested[2].

Species frequencies of four dominant taxa, *C. dutemplei*, *E. umbonifera*, *G. subglobosa* and *O. umbonatus* are shown in Fig. 1*c*. The inferred temperature drop did not affect the relative abundance of three of these species. In contrast, the inferred temperature change seems to have had a pronounced effect on the relative abundance of *E. umbonifera*. This species is the dominant (29–32%) taxa preceding the isotopic event. A rapid decrease in *E. umbonifera* abundance parallels the isotopic curve during the isotopic change. *E. umbonifera* is not present in cores 19–17, and appears again in core 16 (4%). The water-mass event seems to have affected the relative abundance of this species and changed the composition of the assemblage.

Sixteen faunal events occurred before and six after the isotopic event. Using approximate sedimentation rates of 2.2 cm per 1,000 yr for cores 16–21 and 0.5 cm per 1,000 yr for cores 22–24 (ref. 4), the time interval between cores 16 and 20 is about 1.5 Myr, and the interval between cores 20 and 24 is about 5.5 Myr. The interval from cores 20 to 24 is a compressed section relative to cores 16–19, and may explain the greater number of faunal events before the isotopic event.

Only two faunal changes coincided with the development of the psychrosphere. The faunal changes preceding and subsequent to the isotopic change appear to have occurred gradually. This general faunal pattern is observed in other sequences in the Pacific[3] and Atlantic[7,8], where faunal changes are found throughout the late Eocene and early Oligocene.

The development of the psychrosphere in the deep sea created a new physicochemical environment, one of the most widespread in the Cenozoic. The occupation of this niche by new taxa would have either resulted from the migration of taxa into the psychrosphere or from the evolution within the psychrosphere of new taxa. The data from site 277 and from the Pacific[3] and Atlantic[7,8] suggest that the faunal turnover close to the inferred temperature change was limited to a few taxa. Following the development of the psychrosphere, the Foraminifera gradually changed during an interval of several million years until a stable assemblage was established. Some forms were unaffected, being present during both the Eocene and Oligocene. The lack of faunal change occurring immediately after the bottom-water temperature drop suggests that the species must have had fairly wide environmental tolerances, and stenotopic species such as those found at present in the deep sea evolved sometime after the development of the psychrosphere. The stenotopic psychrospheric assemblage probably took a long time to develop, perhaps in response to relatively constant environmental conditions in the deep sea. This interpretation is based on the assumption that the isotopic event reflects a temperature decrease as previously suggested[2,6], and not a change in the oceanic isotopic composition. No faunal change would be expected if the isotopic event reflected a change in the oceanic isotopic composition.

The isotopic data at site 277 show a pronounced cooling in bottom waters occurring in the mid–early Eocene followed by a steady decrease in bottom temperatures to the Eocene/Oligocene boundary[6] where the sharp temperature drop is observed. The decrease in bottom temperatures during the middle–late Eocene may reflect the initial and gradual development of the psychrosphere, with the isotopic change near the Eocene/Oligocene boundary representing part of this development and caused by some event that marks a threshold in Southern Ocean palaeocirculation. If the psychrosphere did develop gradually, this would account for the gradual changes in benthonic Foraminifera observed during the late Eocene before the isotopic drop near the Eocene/Oligocene boundary.

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Late Eocene of Burma yields earliest anthropoid primate, *Pondaungia cotteri*

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In April 1978, a fragment of a primate lower jaw containing the second and third molar teeth was found in late Eocene exposures of the Pondaung Hills about 1 mile north-west of Mogaung village in northwestern Central Burma. This approximately 40-Myr-old specimen is the first fossil primate found in Burma since the fragmentary remains of the controversial earliest anthropoids *Pondaungia cotteri* Pilgrim[1] and *Amphipithecus mogaungensis* Colbert[2] were recovered more than 50 yr ago. The jaw described here is believed to represent further evidence of *P. cotteri*. Its recovery from undoubted late Eocene exposures coupled with its salient higher primate characters and excellent state of preservation provides the opportunity to substantiate further that the Pondaung primates of Burma are the earliest known record of the Anthropoidea.

The new specimen was recovered with a characteristic late Eocene fauna, including fishes, turtles, sebecid and other crocodilians, brontotheres, amynodont rhinoceroses and anthracotheres, as float on greyish yellow, grey and red claystones. These claystones comprise the uppermost 100 feet of the Pondaung Formation near Mogaung village. Locally, the upper beds of the Pondaung Formation are conformably overlain by a southerly emanating marine incursion called the Yaw Formation, containing a rich foraminiferal and molluscan fauna. This fauna has been correlated with the Ludian (=Bartonian) European marine stage[3–7] which dates the underlying Pondaung fauna as late Eocene, approximately 40 Myr BP.

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The jaw fragment (Fig. 1) is part of a right dentary with M_{2-3}, University of California Museum of Paleontology (UCMP) No. 120377, from UCMP locality V78090, Mogaung Northwest. The animal was about the size of the larger species of living gibbons and probably attained a size and weight (based in part on M_2 length correlation[8]) equal to or greater than that suggested for *Aegyptopithecus zeuxis* Simons or *Adapis magnus* Filhol.

We provisionally assign UCMP 120377 to the Anthropoidea and refer it to *Pondaungia cotteri*. Although the fragmentary nature and chemically eroded enamel surfaces of the holotype of *P. cotteri*[1] (Geological Survey of India, GDI D201-203) make comparisons with UCMP 120377 difficult, we feel the referral is justifiable on both morphological and metrical grounds.

Order Primates Linnaeus, 1758
Suborder Haplorhini Pocock, 1918
Hyporder Anthropoidea Mivart, 1864
?Infraorder Catarrhini E. Geoffroy, 1812
Genus *Pondaungia* Pilgrim, 1927
Pondaungia cotteri Pilgrim, 1927

Revised diagnosis: Gibbon-sized primates with bunodont tetracuspate upper molars having weak lingual cingula. The bunodont hexacuspate lower molars have only obscure and incomplete ectocingulids. M_{2-3} with many accessory cuspules and low ridges effecting a strongly corrugated ('pustulate') occlusal surface.
Range: Two localities in the Pondaung Formation, Late Eocene, Central Burma.
Measurements: As given in Table 1.

Table 1 Measurements (mm) of GDI D201 and UCMP 120377

	GDI D201	UCMP 120377
Depth of jaw below anterior root of M_2, external (buccal) surface	11.9	16.3
Depth of jaw below anterior root of M_2, internal (lingual) surface	12.3	17.4
Maximum anteroposterior dimension of crown of M_2	6.1	7.1
Maximum transverse dimension of crown of M_2 (the trigonid is less than 0.1 mm wider than the talonid)	5.9	6.7
Maximum anteroposterior dimension of M_3	7.3	8.3
Maximum transverse dimension of crown of M_3 (= width of trigonid)	5.2	6.0

Immediately recognisable characters of the new specimen are its great jaw depth in relation to molar crown height and its relatively thick mandibular corpus. Nearly all anthropoid primates uniquely share these proportions, which are probably related to increased stresses placed on the mandible during mastication[9,10], and their presence in *Pondaungia* is evidence for its inclusion in the higher primates. Simons[11] made a similar decision regarding *Amphipithecus*. Figure 2 compares the new specimen with *Amphipithecus* (AMNH 32520) and *Homo*. Furthermore, jaw depth in UCMP 120377 is proportionately greater than in the holotype of *Pondaungia* (see Table 1) which could indicate a juvenile status for the type (P. D. Gingerich, personal communication).

M_2 and M_3 of UCMP 120377 are large, bunodont and multicuspate. M_2 is a rectangle with rounded corners in occlusal outline. Unlike most adapines, notharctines and omomyids, the maximum transverse widths of the trigonid and talonid are about equal. In most living and fossil lower primates the talonid is noticeably wider than the trigonid. The trigonid of UCMP 120377 is only slightly higher than the talonid and there is a prominent, closed, highly corrugated talonid basin. This bunodont tooth has a complicated enamel surface with many accessory low cuspules and ridges with no primary trend. Nevertheless, the six principal (and interpretatively primitive) cusps can be identified and delineated (Fig. 1); all are marginally placed. The strong paraconid lies anterolabiad and adjacent to the metaconid, and these two cusps form a twin-peaked crest at the anterolingual corner of the tooth. This arrangement is seen on the M_2 of some omomyids such as *Altanius* Dashzeveg and McKenna[12] and *Tetonoides* Gazin[13] and on some specimens of the early adapids such as *Pelycodus* Cope. The protoconid and hypoconid are, areally, the largest of the cusps and are evenly rounded (that is, symmetrically bunodont). The entoconid is also evenly rounded but smaller than the labial cusps. A faint cingulum lies anterolabiad to the protoconid; otherwise, the lateral faces of the crown of this tooth are smooth.

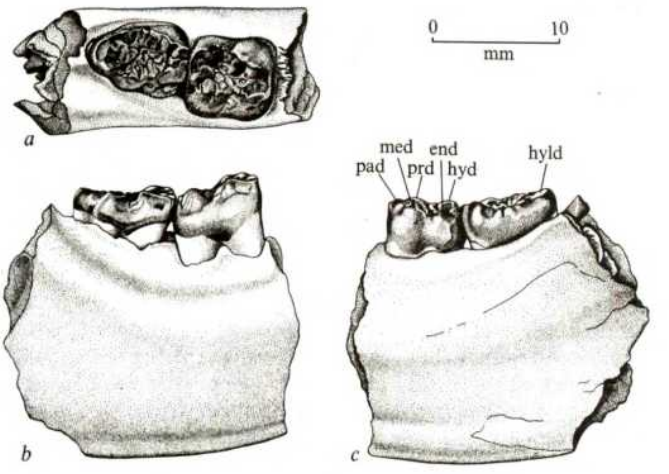


Fig. 1 *Pondaungia cotteri*, right dentary with M_{2-3}, UCMP 120377, from Mogaung Northwest, Central Burma. *a*, Occlusal view; *b*, labial view; *c*, lingual view. Pad = paraconid; end = entoconid; med = metaconid; hyd = hypoconid; prd = protoconid; hyld = hypoconulid.

On the posterior and lateral wall of the M_2 trigonid of *Pondaungia* there is a definable wear facet. This derived (Phase II) wear facet has been called facet 'X' by Kay[14] and facet 11 by Maier[15] and characterises all the living catarrhines and all known fossil catarrhines with the exception of *Oligopithecus*[14]. It has not been found in any Palaeogene lower primate and is absent from all living lower primates with the possible exception of some members of the Indriidae[15].

An area interpreted as a worn hypoconulid is a flat, faceted, transversely elongate eminence at the midline of the tooth, forming the posterior border of the central basin. Before wear, it may have been a twin-peaked cusp. Especially in its abraded state, it is much lower than the hypoconid and entoconid. The presence of a hypoconulid on M_2 is a feature which *Pondaungia* shares with the Fayum and later catarrhine primates (other than Cercopithecidae).

The trigonid cusps and basin of the M_3 are slightly higher than the talonid cusps and basin, as on M_2. Unlike the M_2, however, the trigonid basin of M_3 is separated from the talonid basin by a continuous high-elevation cristid from metaconid to protoconid. As might be expected from a consideration of the lower molars of other Eocene primates, the paraconid of M_3 is smaller, lower and less distinct from the metaconid than on M_2. The metaconid is clearly the highest cusp on M_3. Protoconid and hypoconid are very similar to their homologues on M_2 except that the hypoconid makes a prominent bulge on the labial face of the talonid of M_3, as it does in many other primates. Also, as on M_2, a faint and discontinuous cingulum lies labiad to the protoconid. No

other cingula are present. The talonid area is elongate posteriorly as in other early primates, and the massive, crescentic hypoconulid—well worn on this specimen—forms an elevated posterior border. The central one of three peaks on the lingual marginal ridge of the talonid basin is identified as the entoconid; it is small compared with the entoconid on M_2. The surface of the talonid basin of M_3 is even more intricately corrugated than on M_2. This dental configuration seems best adapted for crushing and grinding soft plant material and fruits.

UCMP 120377 is thus a mosaic of higher and lower primate characters, with the former more predominant and exemplifying the derived (apomorphic) condition. This new specimen is similar in size and general molar features to the early catarrhine *Aegyptopithecus* Simons[16] from the Oligocene of North Africa, but the conical molar hypoconulids and strong cingulid development in *Aegyptopithecus* coupled with the presence of molar paraconids in *Pondaungia* rule out any close relationship. When *Pondaungia* is compared with Fayum parapithecoids *Apidium* Osborn and *Parapithecus* Schlosser, the only marked similarities are the bunodonty and lack of cingulid development shared by *Apidium* and *Pondaungia*.

Comparisons with Eocene primates such as omomyoids or adapoids indicate that the lower-molar patterns of *Pondaungia* (and *Amphipithecus*) could be derived from certain species of *Pelycodus* Cope (Early Eocene, North America and Europe). Pilgrim[1] emphasised this in his description of the type of *Pondaungia*. Furthermore, the upper molars of *Pondaungia cotteri* complement this view, for they seem to have pseudohypocones in the notharctine manner[1]. The more crestiform lower molars of later notharctines in North America and of the adapines in Europe are too specialised (derived) to be considered ancestral, however. Kay[17] also notes that the well developed premetacristid on the M_2 of *Pondaungia* which links up with the paraconid to form a complete medial wall for the trigonid is a feature reminiscent of advanced omomyids and early anthropoids, and further suggests that such a trigonid organisation could rule out known European adapids from the ancestry of *Pondaungia*.

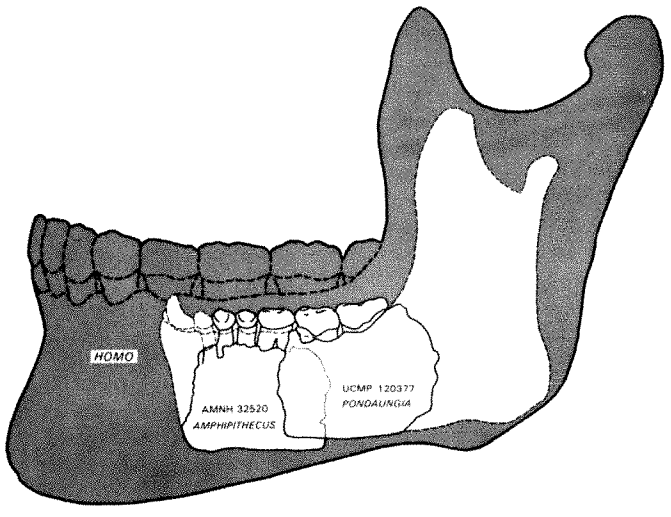


Fig. 2 Size and configuration of the jaw fragments of *Amphipithecus mogaungensis*, AMNH 32520 and *Pondaungia cotteri*, UCMP 120377, compared with *Homo sapiens*. Note how the two Pondaung primates exhibit the same relative jaw proportions as the higher primate *Homo*.

We believe that *Pondaungia* Pilgrim and *Amphipithecus* Colbert are two valid and separate co-existing genera of primates. We also believe that the new specimen of *Pondaungia* was probably a large male in the ancient population that included the type specimen of *P. cotteri* as the smaller females (see Table 1). This conclusion is supported by the differences in size of adult teeth of males and females documented by Swindler[18] for living primates whose overall size is about the same as *Pondaungia*.

We conclude that *P. cotteri* and *A. mogaungensis* were specialised as deep-jawed primates with bunodont cheek teeth, the former being characterised by corrugated enamel surfaces on its teeth, the latter by smooth enamel. Their anatomy indicates they were at or near the ancestry of the anthropoids, perhaps closest to the Catarrhini. Of course, it is possible that they represent a remarkable convergence towards early anthropoids of an adapoid or omomyoid stock. However, we feel that when the anatomical features of *Pondaungia* (and *Amphipithecus*) are considered in the light of their zoogeographic position and latest Eocene age they seem most likely to represent the earliest known record of the Anthropoidea.

Establishing the precise phylogenetic affinities of *Pondaungia* (and *Amphipithecus*) is difficult, in part because of the scarcity of material. As they are now known, both are too aberrant and specialised to be considered ancestral to any known catarrhine. Their characters, however, do not vitiate Szalay's conclusion[19,20] that *Amphipithecus* is an advanced early Tertiary lemuriform–an adapoid. Similarly, neither *Pondaungia* nor *Amphipithecus* falsify Gingerich's conviction (for example, see ref. 21) that adapoids were ancestral to the anthropoids. From another viewpoint, Delson[22] suggests that *Pondaungia* may represent one branch of an omomyid-derived lineage which entered eastern Asia from North America by way of the Bering land bridge; another branch may thence have crossed the Tethys Sea into Africa to give rise to the Fayum (and later) catarrhines. Other hypotheses must be considered, however, pending discovery of more complete fossils from Burma. As the Palaeogene mammalian record of southern Asia is still so poorly known, one might even guess that a primate radicle unrelated to plesiadapiform, adapoid or omomyoid lineages culminated in the late Eocene earliest anthropoids *Pondaungia* and *Amphipithecus*.

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Resistance analysis of sulphur dioxide fluxes to *Vicia faba*

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Before plants respond directly to a gaseous air pollutant, the gas must diffuse into the leaves through stomatal pores or through the cuticle. Some gases are also absorbed or adsorbed on the surface of leaves[1] and this flux elicits no direct plant response. In principle, responses to a pollutant may depend either on the rate at which the gas is absorbed, or on the total quantity absorbed over a period of time. In practice, workers have commonly related responses either to the gas concentration surrounding the plant or to the dose, defined as the product of the gas concentration and the duration of exposure[2]. Plants exposed in an environment where the pollutant flux to leaves is restricted because there is little air movement[3] or because stomata are partly closed, are regarded as having the same dose as plants absorbing the pollutant rapidly. Consequently the response to a specified dose depends strongly on the conditions of exposure[3]. We report here laboratory measurements of fluxes of sulphur dioxide to plants of the field bean, *Vicia faba* L. and relate the observed inhibition of net photosynthesis to this flux. Measured fluxes are analysed to show the several pathways for SO_2 into and on to leaves, and to demonstrate that the internal concentration of SO_2 in the bean leaves was close to zero.

To quantify relationships between concentrations, fluxes and sink strengths, it is convenient to treat the diffusive transfer of gaseous pollutants to plants as analogous to the flow of current in electrical circuits[4,5], a method commonly used in the analysis of water vapour and carbon dioxide fluxes[6,7]. A gaseous flux

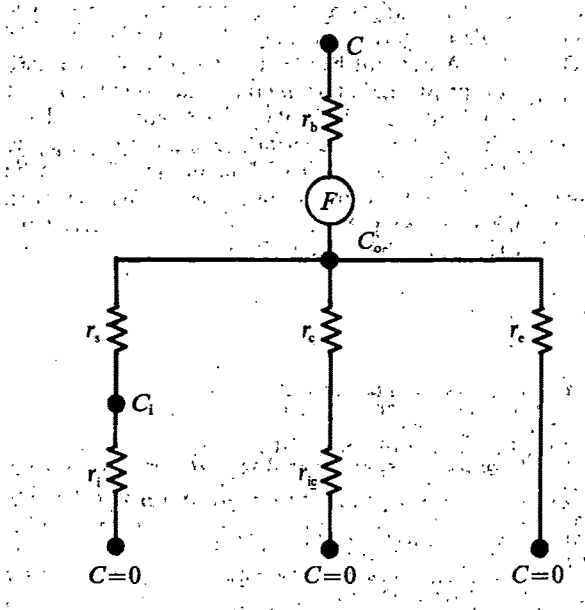


Fig. 1 A resistance analogue of SO_2 uptake by one surface of a leaf. The gas, at concentration C in the exposure chamber, is transferred through a boundary layer resistance r_b to the leaf surface where concentration is C_0. From the leaf surface there are three parallel pathways to sinks where $C = 0$: (1) through stomata, resistance r_s, into the substomatal cavity where concentration is C_i and subsequently through an internal resistance r_i; (2) through the cuticle, resistance r_c and through an internal resistance r_{ic}; (3) by external sorption on the cuticle and on materials on the leaf surface, resistance r_e.

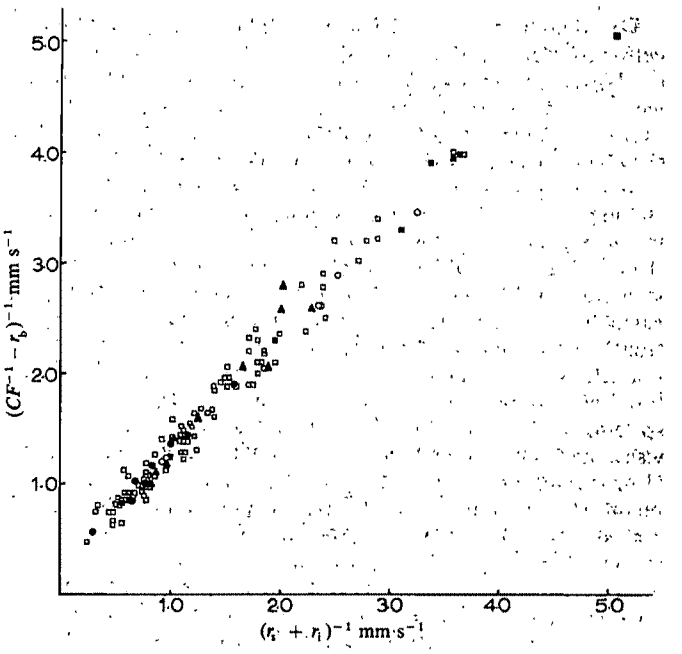


Fig. 2 Variation of $(CF^{-1}-r_b)^{-1}$ with $(r_s+r_i)^{-1}$ for SO_2 uptake by whole plants of *V. faba*. The ordinate is the deposition velocity for SO_2, (flux F/concentration C), modified for boundary layer resistance; the abscissa is the conductance resulting from stomatal (r_s) and internal (r_i) resistances acting in series. SO_2 concentrations: ○, 20 p.p.b.; ●, 40 p.p.b.; □, 75 p.p.b.; ■, 190 p.p.b.; ▲, 300 p.p.b.

(current) is driven by a difference in gas concentration (potential difference) and restricted by diffusive resistances of the leaf boundary layer, stomata and cuticle (resistance). Figure 1 shows a general resistance network for SO_2 uptake by a leaf, indicating parallel pathways through stomata, through cuticle and on to the leaf surface. Inside the sub-stomatal cavity, the SO_2 concentration has the value C_i and further diffusive resistances r_i and r_{ic} describe the restrictions to SO_2 transfer internally as a gas and eventually in solution to a sink where $C = 0$ by definition. In a similar way the resistance r_e limits the rate of surface deposition.

We now describe a method for determining some of the resistances in Fig. 1 from measurements of SO_2 and water vapour fluxes. From Fig. 1, two expressions may be written for the total SO_2 flux density F:

$$F = (C - C_0)/r_b \qquad (1)$$

and

$$F = (C_0 - 0)/[(r_s + r_i)^{-1} + (r_c + r_{ic})^{-1} + (r_e)^{-1}]^{-1} \qquad (2)$$

using the usual laws for finding the resultant of resistances in parallel. Eliminating the surface concentration C_0 from equations (1) and (2) and rearranging terms leads to

$$(CF^{-1} - r_b)^{-1} = (r_s + r_i)^{-1} + [(r_c + r_{ic})^{-1} + r_e^{-1}] \qquad (3)$$

Several terms in equation (3) may be measured or derived. In exposure chambers or cuvettes, the flux density F of gas to unit area of a leaf or plant can be calculated by measuring the concentration difference of gas between inlet and outlet of the chamber and the gas flow rate[8]. The gas concentration C is easily measured. Values of boundary layer and stomatal resistances, r_b and r_s, for SO_2 transfer may be found from analyses of water vapour loss from real and model leaves, making allowances for differences in diffusivity between SO_2 and H_2O (refs 5, 9). The form of equation (3) shows that if measured values of $(CF^{-1} - r_b)^{-1}$ are plotted against $(r_s + r_i)^{-1}$, a straight line graph with slope unity and intercept $[(r_c + r_{ic})^{-1} + r_e^{-1}]$ should result. Because r_i is not known *a priori*, it must be estimated, assuming that it is independent of F and r_s, to obtain the best straight line from a set of measurements.

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We measured fluxes of SO_2 to whole plants of *Vicia faba* cultivar Dylan exposed, when they had three pairs of leaflets, in a cubical chamber (side 0.5 m) described elsewhere[9]. The plants were in pots with the soil surface covered to prevent gas exchange. To minimise SO_2 uptake on chamber walls and pots, they were covered with polyvinyl fluoride film and allowances were made in analysing the results for small residual SO_2 fluxes to the film and uncovered plant stems. Simultaneous measurements of concentration differences of SO_2, H_2O and CO_2 across the chamber allowed fluxes of these gases to be determined and stomatal resistances and rates of net photosynthesis were calculated[9,10]. The mean boundary layer resistance to SO_2 transfer in our experiments was 100 s m^{-1}.

Each plant was allowed to acclimatise for one photoperiod in the chamber; on the second day, photosynthesis and transpiration in clean air were measured, and on the third day SO_2 was added at fixed concentrations ranging from 20 to 300 p.p.b. (1 p.p.b. = 10^{-3} p.p.m. $\hat{=}$ 2.86 μg SO_2 m^{-3}). To change stomatal resistance, irradiance was varied using neutral filters. Values of r_s ranged from 200 to 3,700 s m^{-1}. Throughout the experiments air temperature was 20°–21 °C and relative humidity was 50–60%.

Figure 2 shows the values of $(CF^{-1}-r_b)^{-1}$ plotted against $(r_s+r_i)^{-1}$ when r_i was assumed zero. Regression analysis shows that the points are well fitted ($r = 0.99$) by a straight line with slope 1.02 ± 0.01 and intercept 0.28 ± 0.02 mm s^{-1}. The reciprocal of the intercept, 3,600 s m^{-1} corresponds to the resultant of r_e and (r_c+r_{ic}) in parallel (Fig. 1 and equation (3)) and it is not possible to calculate the values of these resistances independently from these measurements. However, Spedding[1] found that r_e for surface deposition of SO_2 on filter paper ranged from 2 to 5×10^3 s m^{-1} and if r_e is similar for bean leaves we conclude that (r_c+r_{ic}) must have been of the same order of magnitude as r_e to give a resultant of 3,600 s m^{-1}.

Further analysis of our measurements with different values assumed for r_i reveals that the uncertainty in the estimate of r_i is about 20 s m^{-1}, an order of magnitude smaller than the minimum stomatal resistance we observed. At a typical value of $r_s = 300$ s m^{-1}, analysis of the resistance network in Fig. 1 for the resistances derived here suggests that about 90% of the total SO_2 flux to the leaves entered the stomata and 10% was deposited on the surface and/or diffused through the cuticle. Although this latter figure was small in our experiments, it would be necessary to find out how surface and cuticular resistances are influenced by natural 'weathering' and other environmental factors in the field before total deposition of SO_2 on vegetation could be estimated reliably.

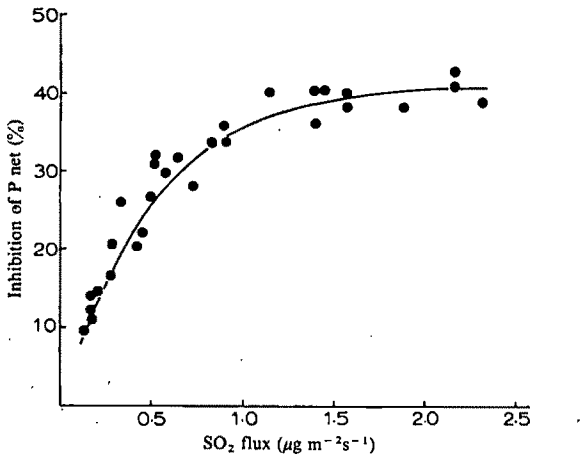


Fig. 3 Variation of the percentage inhibition of net photosynthesis in *V. faba* by SO_2, relative to clean air, with SO_2 flux entering through stomata. Plants were exposed to SO_2 concentrations ranging from 20 to 200 p.p.b. and the maximum depression in photosynthesis over 2 h was measured.

When plants of *Vicia faba* are exposed to SO_2, rates of net photosynthesis decrease relative to rates observed in clean air[10]. Figure 3 shows the percentage inhibition of net photosynthesis as a function of SO_2 flux density into stomata. In these measurements, plants were exposed for 2 h to SO_2 at concentrations ranging from 20 to 200 p.p.b., and the maximum decrease in net photosynthesis at light saturation was recorded. Fluxes of SO_2 into the stomata were calculated from equations (1) and (2) assuming $r_i = 0$.

By relating response(s) to pollutant flux rather than dose, the influence of different stomatal, surface and boundary layer resistances in different experimental systems is removed. Comparisons of response when fluxes are known may reveal better agreement between responses observed in different laboratories than is apparent from current literature. However, further work is necessary to determine whether specific responses depend on rates of uptake, on uptake integrated over long periods, or on more complex definitions of exposure.

Our measurements and analysis show that at the low and medium concentrations of SO_2 we used, the internal resistance to SO_2 uptake in *Vicia faba* was negligibly small. Consequently fluxes into plants could be estimated from the external SO_2 concentration and the appropriate stomatal resistance. If this result holds generally, the uptake of SO_2 by vegetation in the field or laboratory can be estimated either by relatively simple direct measurements of stomatal resistances and gas concentration or by reference to the extensive literature describing stomatal resistances of many natural and agricultural species. At the biochemical level, our finding that the internal resistance to SO_2 uptake was very small has significant implications for the mechanisms of action of SO_2 within plants.

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'Helpers' in fox society

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Among the Carnivora the presence of non-breeding adults within social groups seems to be widespread, and evidence is accumulating that these individuals sometimes contribute to the survival of young born to other group members who may, or may not, be their relatives[1,2]. I describe here studies of both wild and captive red foxes, *Vulpes vulpes*, and report that non-breeding vixens occur in fox groups in some habitats. These barren vixens are subordinate to the breeding females, and may feed, guard, groom and play with the cubs of the breeding vixen within their group.

The social organisation of the red fox is still poorly understood, but it is certain that it varies between habitats and sometimes involves monogamous pairs and elsewhere small family groups, and that the proportion of barren vixens also varies between habitats[3,4]. Around Boars Hill, Oxford, fox groups comprise four or five adults, normally including one male

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and several related females, who share a group territory[5]. Of four groups studied in detail in 1973–74, 60% of the vixens were barren, that is, only one or two vixens had cubs in each group. Observations of encounters between barren and reproductive vixens within each group suggested that the breeding vixens were generally dominant amongst the adult vixens of their group. Radio-tracking and direct observation showed that some barren vixens visited breeding earths where they were frequently seen playing with cubs, and circumstantial evidence suggested that they also provided food for the cubs. Where two litters are born within one territory, they may be communally reared in the same earth.

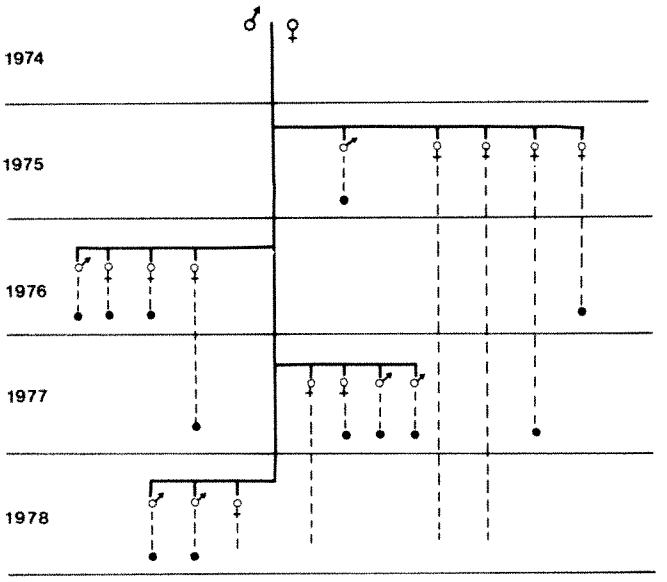


Fig. 1 The genealogy of a captive group of foxes (group I), showing that during 1974–78 only one vixen bred. ● Indicates that individuals were removed from the group. Some non-breeding females acted as helpers.

To study fox reproductive behaviour, two family groups, each mimicking the composition of wild groups, were watched in 1,000-m^2 enclosures. Group I grew from a pair and their offspring; group II grew from a male and three full sisters of the same age. Hence, both groups comprised related animals. During 1974–79, the groups' composition changed with the addition of cubs each year. All subadult males were removed each autumn, as were some vixens of randomly selected ages.

In eight cumulative years of study of the two groups, the maximum productivity would have been 27 litters. In fact, only seven litters were born (32% productivity, see Fig. 1). Every year except one, only one vixen gave birth to cubs in each group and in every year only one vixen's cubs survived in each group. The male ignored the other females during the mating period and their oestrous condition could not be determined externally. In group I the same vixen bred for 5 yr, throughout which she was consistently dominant to all the others. In group II only one vixen bred in 3 out of 4 yr and two bred in 1 yr, but the identity of the breeding individual changed from year to year. However, in every case the breeding vixen was dominant to the others during the winter in which she bred (see Fig. 2).

In each group some of the non-breeding vixens visited the newborn cubs frequently, but briefly, for the first 3 weeks of their lives; thereafter, they behaved in a way which might promote cub survival by providing food, security and grooming (Table 1). They also attacked wild foxes outside the enclosures while the mother was tending to her cubs, or heavily pregnant. As cubs grew, the litters were split so that some cubs rested with their mother while others rested with non-breeding females. Table 1 shows that each member of a captive family group often participated in tending to the cubs. However, the percentages given do not necessarily numerically reflect the benefit to the cub that accrued from the behaviour of a particular adult, nor the costs to the adult of its behaviour. This is because the adults did not all 'help' at the same time, nor did they have equal access to the resources they gave to the cubs.

In 1976, the breeding vixen of group 1 suffered a serious injury to a front paw when her cubs were 4 weeks old. On only 2 out of 16 days following the injury was the vixen able to tend to her cubs, but during this period they were found with non-breeding vixens, with whose care they all survived.

In the winter of 1977–78 in group II, the dominance of vixen WP over vixen BE waned, so that by January 1978 they were of equal status (assessed by frequency and direction of amicable and aggressive interactions). Perhaps as a consequence of their equal status, both vixens conceived. By the time the cubs were born, BE was clearly dominant to WP. In the previous year WP had been a successful mother while BE had been a diligent 'helper'. This year BE's cubs were born 13 days before those of WP. During those 13 days BE tended her own cubs. When WP's cubs were born she (WP) repeatedly picked them up and carried them around the enclosure, giving submissive shrieks and adopting submissive posture. Sometimes this 'panic' seemed spontaneous but on other occasions it was prompted by BE's approach. WP's cubs soon succumbed to this treatment. The death of the last cub at 8 days old was followed by a sudden change in WP's behaviour; dropping the corpse outside BE's artificial earth, WP entered and submitted to BE. During the next 10 min WP visited BE's cubs 10 times (having not done so since the birth of her own cubs), and BE visited WP's now empty earth three times. On return from the third trip BE found WP suckling her cubs; the two vixens greeted and BE wandered off leaving WP with her cubs. WP's agitated behaviour had disappeared completely. The two vixens reared BE's cubs cooperatively thereafter.

Table 1 Behaviour of each member of a family group of foxes towards a litter of cubs

	Father Sm	Mother WP	Aunt BE	Aunt WE
Provide food $n = 151$	41.1	21.2	17.9	19.9
Guard $n = 82$	2.4	25.6	61.0	11.0
Retrieve $n = 30$	13.3	43.3	40.0	3.3
Groom/play $n = 139$	34.5	12.9	32.4	20.1

Values given are the percentage of each of four categories of behaviour undertaken by each member of the group towards the litter (born 19 March) between 5 April, when the cubs began to eat meat, and 18 June 1977. The behaviour categories are providing food, guarding in the earth, retrieving cubs straying from the earth and grooming or playing with cubs.

Each year, in the first 3 weeks following the birth of her cubs, the dominant vixen, irrespective of her identity, furiously attacked the most subordinate of the other vixens, although amicably greeting the more dominant of the subordinates. Each year the more dominant of the subordinate vixens began to feed the cubs earlier than did the more subordinate females. As they began to feed the cubs, each helper suddenly gained 'confidence' during encounters with the male fox. The male frequently played with the cubs and never seemed to threaten their safety. He regularly fed the cubs and, from the moment of their birth, he both fed their mother and cached food around the natal earth.

The female helpers never fed the mother, only the cubs. In spite of their otherwise similar behaviour, foxes apparently differ from jackals[1] in two important respects; jackal helpers feed the breeding female, and male and female jackals are equally likely to act as helpers, whereas most (perhaps all) young male foxes leave the group in their first year[6,7].

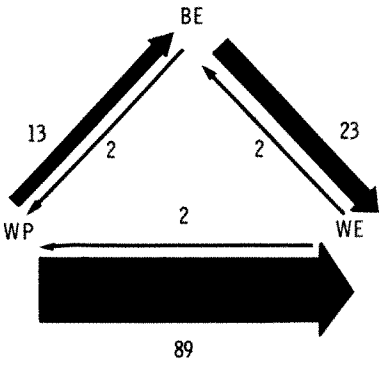


Fig. 2 The number and direction of aggressive interactions between three full sisters within a family group of foxes during the 1977 breeding season (proportion of interactions indicated by arrow thickness). Of 660 interactions recorded between group members, 131 involved aggression between these three females. On this basis a linear hierarchy of WP, BE, WE can be seen.

The mechanism underlying the male foxes' lack of sexual interest in non-breeding vixens is unknown. Two ideas could explain why in the wild the barren vixens remain in the group where they do not breed but do apparently contribute to the survival of another individual's offspring. The helpers may be 'hopeful reproductives'[8], maintaining a group structure and territory which confer benefits which may at some future time be theirs. The second possibility is that the helpers gain through direct increase in their inclusive fitness (kin selection)[9] by increasing the chances of survival of cubs to which they are related. These two hypotheses are not mutually exclusive and their relative importance depends, in practice, on the extent to which female membership of fox groups is restricted to relatives. The evidence so far suggests that immigration into established groups is not common and, because helpers could simultaneously be relatives and 'hopeful reproductives', there is no way of deciding whether one or both explanations is correct.

Post-mortem studies of fox reproduction in various habitats have shown differences in the proportion of barren vixens between areas and from one year to the next[3,10] and between 'age classes'[11]. These studies suggested that breeding is influenced by density-dependent factors. Reproductive suppression of subordinate vixens in those areas where ecological circumstances permit the formation of multi-female groups could be just such a factor.

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A unique form of locomotion in a stomatopod—backward somersaulting

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Of the diversity of forms of locomotion evolved by life on Earth, one that seems not to have developed to any great extent is rolling or somersaulting. Many cultures claim the existence of 'hoop snakes' that grasp their tails in their mouths and roll downhill, but no known snake can perform this feat[1]. Furthermore, although at least one spider (Hamilton, personal communication) when disturbed forms its body and legs into a sphere and rolls down sand dunes, I have been unable to find examples of animals that roll uphill or even over level terrain. Various coelenterates such as *Hydra* and some sea anemones somersault slowly across the substrate by alternating attachment of the pedal disk and tentacles. However, with the exception of human gymnasts and other playful primates, I report here a unique form of locomotion found in the stomatopod *Nannosquilla decemspinosa* (Rathbun, 1910) that consists of a rapid series of backward somersaults.

N. decemspinosa is a small stomatopod growing to a maximum body length of 23 mm. This predatory marine crustacean is found in sand substrates on the Pacific coast of Central and South America. The population studied occurred in the lower third of the intertidal zone on a sand beach on Naos Island, Canal Zone. Individuals excavate 10–15-cm vertical burrows stabilised by a mucus secretion mixed with the sand. When covered by water, the animals sit at the entrance with only their eyes and antennules protuding, waiting for passing prey. When their burrows are exposed at low tide, *N. decemspinosa* close the entrance with a few sand grains and retreat to the bottom of the burrow. *N. decemspinosa* rarely leave their burrows except to dart out and seize prey (usually less than one body length), to locate mates, and when strong wave action destroys their dwelling. When out of the burrow in water, they almost always swim using their five pairs of abdominal pleopods for propulsion. They seldom take more than a few steps on the substrate. One exception is during burrow construction, when they will carry sand 1 or 2 cm from the entrance. Inside the burrow, they use their laterally directed walking legs, as well as their pleopods, in moving quickly up and down. At low tide, if exposed by wave action, they swim if there is as little as a millimetre of water. However, if the sand is well drained and only moist, they are incapable of walking and use a radically different form of locomotion—backward somersaulting. The animal rolls over on its back and brings the telson up over its body, planting it immediately in front of the head, forming a nearly closed loop. Waves of flexion then pass anteriorly from the posterior abdomen and another from the posterior thorax, rolling the animal forward. Finally, the head and carapace are pulled up over the rest of the body and flip over the top so that the animal is once again lying stretched out on its back. At this point the telson is again flexed anteriorly and a new cycle begins (Fig. 1).

The animal continues a series of backward somersaults, generally moving in a straight line until it encounters an obstacle or water sufficiently deep for swimming, or until it becomes fatigued. Although the longest track recorded was just over 2 m, most animals travelled somewhat less than 1 m. The distance covered by each somersault is basically the length of the body. As the average adult is 20–23 mm long, the animals made 20–40 consecutive somersaults.

Body length and speed were positively correlated ($r = 0.857$, $n = 30$, $P < 0.001$) (Fig. 2). Thirty animals ranging in length from 12 to 23 mm were brought into the laboratory. Each was

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placed on a level, damp sand substrate and the total length and time taken for a somersaulting track recorded. The sand surface seemed identical to that of the beach at low tide. Animals were started by dropping them onto the sand from a height of 5 cm. All immediately began somersaulting. Curved tracks and those shorter than 15 cm were not scored and the animals were retested several minutes later. In this series of tests, speeds ranged from 1.5 to 4.5 cm s^{-1}. In other trials, the maximum speeds recorded were 5.6 cm s^{-1} for a 20-mm female and a 23-mm male.

To determine whether the animals take any particular orientation with respect to the beach when somersaulting, several individuals were dug out of their burrows at low tide (during both the day and the night) and were dropped immediately onto smooth sand near their burrow. The initial direction taken was then noted. Direction taken was not significantly different from random orientation, although there was a tendency for animals starting uphill to curve more often than those heading downhill. However, *N. decemspinosa* could somersault up slopes as steep as 10°, and in the laboratory on a wooden substrate some animals could climb even a 30° incline.

This unique form of locomotion in *N. decemspinosa* seems to have evolved in conjunction with other morphological adaptations related to inhabiting fine sandy substrates. The *Nannosquilla* body type is among the most slender, dorsoventrally flattened and loosely articulated of any stomatopod[2]. This allows it to use small-diameter burrows requiring a minimum of time, effort and stabilising mucus for construction. It is particularly important to *N. decemspinosa*, as this species frequents sandy beaches where burrows are commonly destroyed by wave action. *N. decemspinosa* also is capable of making jack-knife turns in a tube barely larger in diameter than its own width. When digging, it proceeds to the bottom of its burrow headfirst, grabs a load of sand in its maxillipeds, makes a U-turn and carries the sand to the surface. Thicker and more tightly articulated stomatopods, such as the cavity-living gonodactylids or squillids inhabiting U-shaped burrows, cannot turn 180° in such narrow quarters and would require a relatively larger diameter burrow.

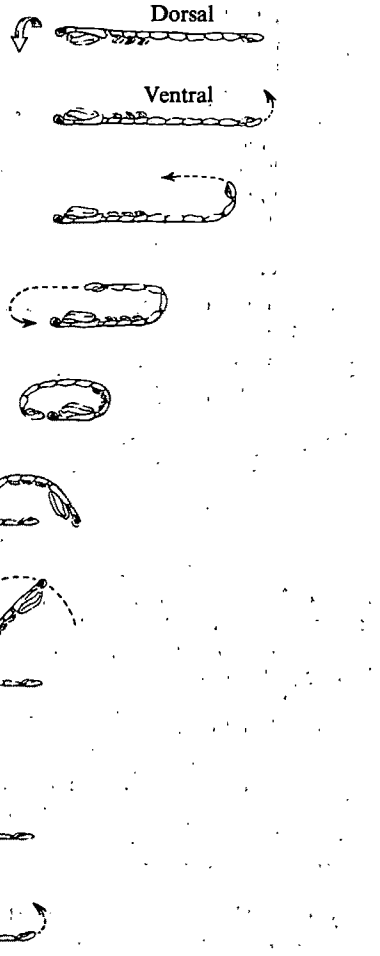


Fig. 1 One cycle in the backward somersaulting of the stomatopod, *N. decemspinosa*. If the animal is dorsal side up, it rolls over onto its back. It then draws the telson up over its head, rolls forward until the head and thorax are flipped up over the body, and lands with the body again stretched out on its back. Animals may complete 20–40 consecutive somersaults.

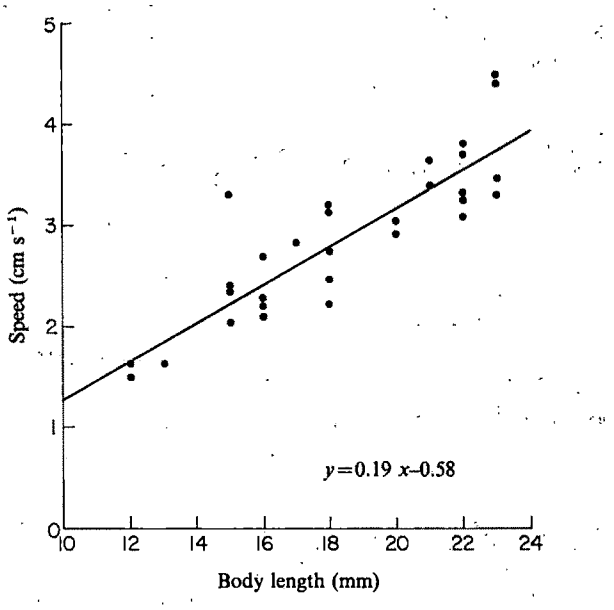


Fig. 2 Speed of backward somersaulting as a function of body length in *N. decemspinosa*. Each point represents a single individual somersaulting on smooth sand. Body length and speed were positively correlated ($r = 0.857$, $n = 30$, $P < 0.001$).

Most intertidal stomatopods, when exposed on dry land, are capable of crawling using their walking legs. A few can also inch their way forward using their spined telsons to push themselves along. The long, slender body of *N. decemspinosa* offers too much resistance in contact with the substratum for its legs to pull it forward, and the laterally directed legs are too short and weak to lift the body off the sand to reduce friction. The alternative method is somersaulting. Although the turning movements used in the burrow are different from those used while somersaulting (turns in the burrow are made headfirst while somersaults are telson-first), the flexibility essential for making U-turns in the burrow is also required for somersaulting. Also, the dorsoventral flattening provides a broad, flat dorsal surface that gives the animal considerable stability while rolling. Most stomatopods have more rounded dorsal surfaces in cross-section. Thus, although the evolution of the long, slender body makes it impossible for *N. decemspinosa* to move on dry surfaces by walking or crawling, it does provide the flexibility needed for somersaulting.

The data suggest that there is no initial orientation with respect to the slope of the beach during somersaulting. However, the animals are proceeding in an anterior direction and can see objects in their path. Several times, somersaulting animals stopped before running into objects, but there was no indication that they could alter their course to avoid obstacles. When animals were rolling along parallel to the beach, they frequently curved downhill towards the water. This was presumably due to gravity pulling them in that direction, much as a hoop rolled across a slope will curve downhill. Thus, although animals somersault in any direction, even uphill, and do not seem to orientate in any particular direction, there is a tendency to move down the beach towards water. As they are

most likely to be exposed by wave action when the tide is coming in or going out, movement of only 1 or 2 m down the beach would probably bring them back into contact with water.

I have observed one other species of *Nannosquilla* (not described from the Atlantic coast of Panama), and it also somersaulted when exposed. Similar morphology in other *Nannosquilla* species would suggest that they may also possess this ability. Interestingly, another species of stomatopod, *Acanthosquilla digueti*, that is found in micro-sympatry with *N. decemspinosa* and has a very similar body shape, does not somersault. When exposed, it turns over on its back as does *Nannosquilla* and flips its telson up over its head, but it does not continue the motion completing the somersault, and simply flops back and forth. I have never seen it make two consecutive somersaults. Whether other lysiosquillids with similar morphologies can locomote by somersaulting is unknown.

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Protective immunisation of mice using cell surface glycoprotein from *Trypanosoma cruzi*

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The parasitic protozoan *Trypanosoma cruzi* is the causative agent of the chronic debilitating Chagas' disease in man. It is estimated that at least 12 million people in South and Central America are infected with *T. cruzi*, for which there is so far no suitable chemotherapy[1,2]. The development of vaccines using killed or attenuated whole organisms is hindered by the presence of endotoxin-like material in the protozoa[3], and the possible existence of antigens in *T. cruzi* which cross-react with, and thereby stimulate immunity against, mammalian heart and nervous tissue[4,5]. Because autoimmunity induced in this way has been thought to cause the cardiac and neural lesions characteristic of the chronic stage of the disease[6,7], it has been assumed that immunisation based on a whole organism vaccine might carry the risk of itself inducing symptoms of the disease. Recently an integral membrane glycoprotein of molecular weight 90,000, was isolated from the cell surface of *T. cruzi*. It is the only major cell surface glycoprotein, it is present throughout the life cycle of the parasite[8], and it does not undergo antigenic variation[9]. We have successfully protected mice against acute lethal *T. cruzi* infection by vaccinating them with glycoprotein in combination with adjuvants. Moreover this glycoprotein does not seem to be the one carrying the antigens causing autoimmune antibody formation.

The prophylactic immunisation of mice with *T. cruzi* glycoprotein in conjunction with either Freund's complete adjuvant or saponin as adjuvant against a subsequent lethal challenge with Y strain *T. cruzi* is illustrated in Fig. 1. (The successful use of saponin as an adjuvant for immunising mice with various whole *T. cruzi* vaccines has been described previously[10].) Unimmunised C57BL/6 mice (Fig. 1*a*) all developed high blood parasitaemias and died (mean survival time, MST, 24.2 ± 0.75 days). Immunisation with Freund's complete adjuvant alone was not protective as judged by either blood parasitaemia or survival time (MST 25.2 ± 0.5 days). After immunisation with glycoprotein alone all mice died but parasitaemia was delayed and

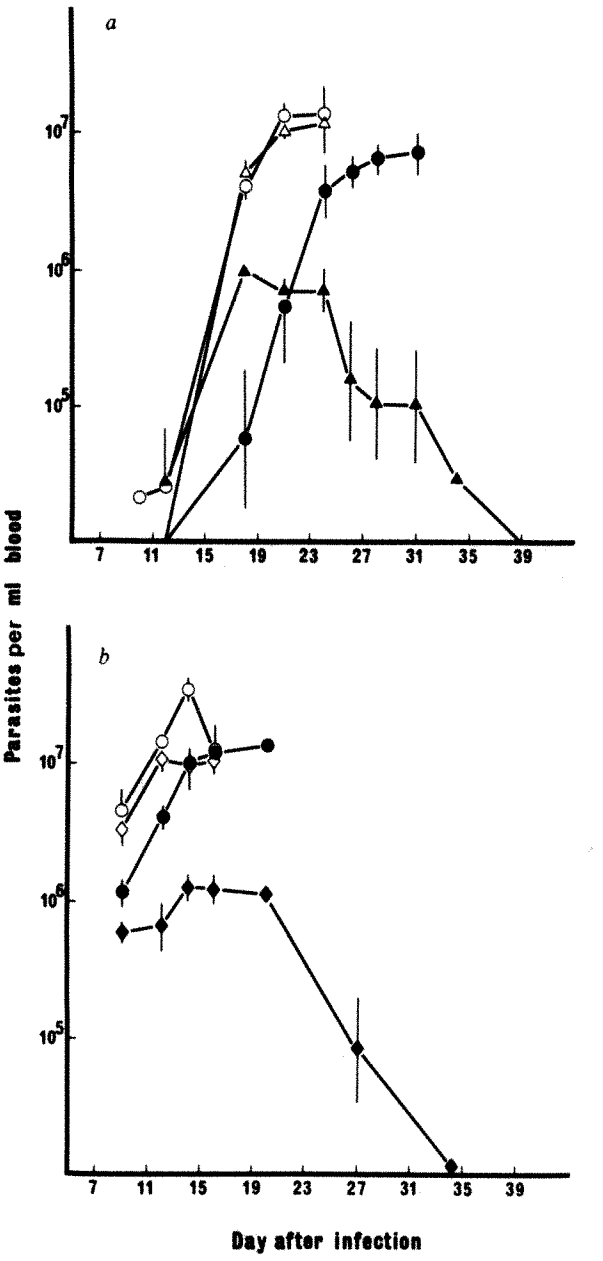


Fig. 1 Protection of mice against acute lethal *T. cruzi* infection by immunisation with *T. cruzi* glycoprotein and adjuvant. *a*, Female C57BL/6 mice were immunised s.c. with 100 μg glycoprotein emulsified in an equal volume of Freund's complete adjuvant (Difco) (▲), glycoprotein alone (●) Freund's complete adjuvant alone (△), or were untreated (○). Immunisation was repeated after 14 days and, 14 days after the second injection, all mice were challenged s.c. with 5×10^3 Y strain blood trypomastigotes from mouse passage. At the times shown, 10-μl blood samples were taken, mixed with 0.87% ammonium chloride, and parasites counted using a phase contrast microscope. There were five mice in each group and the vertical lines shown represent ± s.e.m. *b*, Female (CBA × C57BL/6)F_1 mice were immunised as in (*a*) except that 50 μg of *Quillaya* saponin (SPL Boake Roberts and Co.) was substituted for Freund's complete adjuvant. Mice were challenged s.c. with 7.5×10^4 Y strain trypomastigotes. ◆, 100 μg glycoprotein plus 50 μg saponin; ●, glycoprotein alone; ◇, saponin alone; ○, untreated control. There were five mice in each group and the vertical lines represent ± s.e.m. Glycoprotein: for the above experiments a clone of epimastigotes (Wel Tryp Y_2-C_1) was used as a source of glycoprotein. Culture conditions and glycoprotein preparation using the lectin from *Lens culinaris* for affinity chromatography were described previously[8] except that the non-ionic detergent Renex 30 (Honeywell Atlas) was used to solubilise the glycoprotein and throughout the fractionation procedure. Glycoprotein was precipitated by adding 3 vol. of ethanol and incubated at −20 °C for 48 h. The precipitated glycoprotein was resuspended in phosphate-buffered saline for immunisation.

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Table 1 Absorption of autoreactive human Chagas' serum with whole *T. cruzi* or glycoprotein fraction

Serum	IFA titre against Heart*	*T. cruzi*†
Chagas' serum unabsorbed	1:512	1:4,096
Chagas' serum absorbed *T. cruzi*‡	1:64	1:8
Chagas' serum absorbed glycoprotein§	1:512	1:1,024
Normal human serum	1:8	1:16

* IFA (indirect fluorescence assay) for anti-heart reactivity as for Fig. 2.

† Air-dried smears of *T. cruzi* epimastigotes from *in vitro* culture were substituted for heart in IFA.

‡ Absorbed with 45 mg lyophilised epimastigotes ($\simeq 4.5 \times 10^9$ organisms per ml serum; 1 h at 37 °C and then overnight at 4 °C.

§ Absorbed with glycoprotein (500 μg protein, $\simeq 10^{10}$ organisms per ml serum; 1 h at 37 °C and then overnight at 4 °C.

survival prolonged (MST 31.2 ± 1.2 days). The combination of glycoprotein with Freund's complete adjuvant significantly reduced parasitaemia levels and allowed all mice to eliminate parasites from the blood, as judged by microscope count, and survive. The same pattern was seen in (CBA × C57BL/6) F_1 mice where saponin was substituted for Freund's complete adjuvant (Fig. 1*b*): mice immunised with glycoprotein in conjunction with saponin all cleared their blood parasites and survived whereas those receiving either glycoprotein or saponin alone died with high blood parasitaemias. In the experiment shown the degree of protection afforded by immunisation with glycoprotein alone was less than seen in the previous experiment (Fig. 1*a*), however, in other experiments it has been more marked. It is clearly variable and, being relatively weak, its protective capacity probably depends on the virulence of the individual experimental challenges.

A characteristic manifestation of the autoimmunity associated with Chagas' disease is the development of autoantibody directed against endocardium, vascular and interstitial tissue in mammalian heart (EVI antibody[4]). To determine whether the glycoprotein antigens shared identity with the tissue antigens against which *T. cruzi*-induced autoimmunity is directed, serum from a human Chagas' disease patient showing characteristic EVI activity (Fig. 2) was absorbed with glycoprotein. Table 1 shows that whereas absorption of serum with lyophilised whole *T. cruzi* epimastigotes significantly reduced anti-EVI activity and completely abolished anti-*T. cruzi* activity, absorption with glycoprotein did not affect the anti-EVI titre and was less effective at reducing anti-*T. cruzi* activity. These data indicate that the glycoprotein and the relevant tissue antigens do not cross-react, and also confirm that the glycoprotein represents only a part of the antigenic profile of the whole organism. Five further sera from human Chagas' disease patients (mean EVI titre $\log_2 6.7 \pm 0.4$) have been similarly absorbed and again absorption with glycoprotein did not significantly reduce EVI activity (titre $\log_2 6.2 \pm 0.5$), whereas absorption with whole *T. cruzi* epimastigotes did (titre $\log_2 4.3 \pm 0.8$).

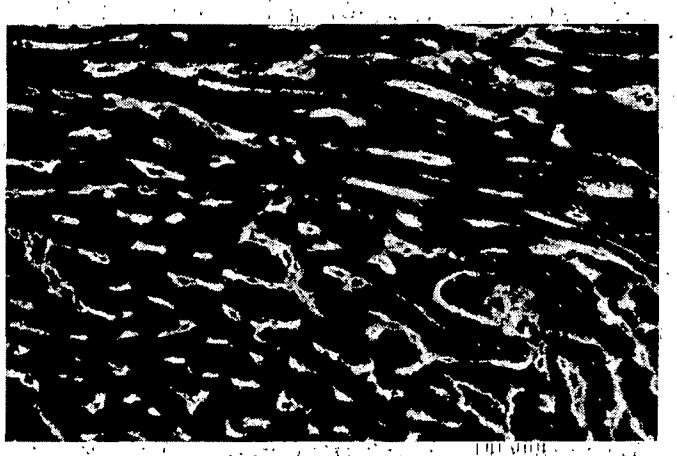


Fig. 2 Characteristic EVI reactivity of human Chagas' serum against mammalian heart tissue. Cryostat sections of mouse heart (5 μm thick) were prepared and stored at −20 °C. They were incubated with serial dilutions of test serum for 20 min at room temperature, washed, developed with a 1 in 20 dilution of FITC-labelled rabbit anti-human IgG (Miles Yeda) for 20 min at room temperature and, after a final wash, examined using a fluorescence microscope. Normal human serum in identical conditions gave no detectable staining.

These results together with the protection data suggest that the glycoprotein could be a candidate antigen for a vaccine against American trypanosomiasis. Immunisation with glycoprotein alone was partially protective as evidenced by slight, but significantly, lower blood parasitaemias and prolonged survival times. Further research should establish whether the adjuvant requirement is absolute for optimal immunisation with the glycoprotein and, if so, whether clinically acceptable adjuvants may be substituted. We also require to know whether any glycoprotein-based vaccine is capable of achieving sterile immunity, and being prophylactically effective against later development of chronic forms of Chagas' disease. It has been proposed that the late pathological manifestation of chronic Chagas' disease develops as a result of tissue damage inflicted during the acute phase of infection[11]. Any reduction in the acute phase may, therefore, in addition to reducing the mortality associated with acute infection, also decrease the incidence or severity of the chronic form of the disease.

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Interferon enhances 2-5A synthetase in embryonal carcinoma cells

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Mouse teratocarcinomas provide a useful model of mammalian differentiation, because the malignant embryonal carcinoma (EC) stem cells of such tumours may produce various differential cell types *in vivo* or *in vitro*[1]. Many EC cell lines have now been established and classified on the basis of their ability to differentiate *in vivo* into cell types characteristically derived from any of the three germ layers[2,3]. There is convincing evidence that EC cells can neither produce interferon, nor respond to it by becoming resistant to virus, whereas differentiated cells derived from EC lines behave normally in both respects[4]. We investigated the lack of responsiveness of EC cells towards interferon by measuring the levels of two double-stranded RNA -dependent enzyme activities recently shown to be enhanced by interferon[5,6].

We report here that on treatment with interferon, EC cells show increased 2–5A synthetase levels comparable to those found in differentiated cells, while there is little or no effect on kinase activity in EC cells, in contrast to their differentiated counterparts.

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The conditions of culture and sources of the cell lines used have been described elsewhere[7,8]. Briefly, all the EC cell lines used are derived from the transplantable ascitic form of the OTT 50-60 teratocarcinoma isolated by Stevens[9]. PCC3/A/1 and PCC4AzaR1 are multipotent lines which give rise to a variety of differential cell types *in vivo*, and in appropriate conditions *in vitro*[8]. The line 3/A/1-D-1 M is a cloned differentiated derivative of PCC3/A/1. These non-malignant cells characteristically give rise to bone *in vivo*. As well as this line, mouse L929 cells were used as differentiated controls which respond to interferon by the establishment of antiviral state.

The first 2-5A synthetase, forms oligonucleotides of the series pppA(2'p5'A)n—collectively referred to as 2-5A—which potently inhibit protein synthesis *in vitro* and *in vivo*, probably by activation of a nuclease[10–14]. The kinase studied, phosphorylates a protein of molecular weight 67,000, a 38,000-MW protein which is almost certainly the small subunit of initiation factor eIF2, and exogenous histones[5,15–18].

The 2-5A synthetase and the protein kinase were assayed after partial purification on columns of poly(I) . poly(C)–Sepharose as elsewhere described[5,6]. The column-bound synthetase is stable, thus providing a convenient method for the synthesis of 2-5A free of the enzyme responsible for its degradation[5]. Similarly, the poly (I) . poly(C)–Sepharose-bound protein kinase is stable and free of any phosphatase activity which may be present in the cell extracts[5].

Figure 1 shows a typical comparison of the kinase activity in PCC3/A/1 cells and their differentiated derivatives 3/A/1-D-1 M, together with L929 cells with and without interferon treatment. The enhanced phosphorylation of the 67,000-MW polypeptide is apparent in cell fractions from L929 and 3/A/I–D–1 M cells (Fig. 1, lanes 2, 6) but not from PCC3/A/1 cells (Fig. 1, lane 4). As a test for enhanced kinase activity, phosphorylation of calf thymus histones was analysed by the poly(I) . poly(C)–Sepharose-bound enzyme fractions. As expected of interferon-treated cell extracts, there was an enhanced phosphorylation of one of the major histone bands in fractions from L929 and 3/A/1-D-1 M cells (Fig. 1, lanes 8, 12) but not from PCC3/A/1 cells (Fig. 1, lane 10). Phosphorylation of the 67,000-MW protein and added histone by the poly(I). poly(C)–Sepharose-bound enzyme(s) fractions (Fig. 1) from extracts of cells untreated with interferon was also apparent although to a lesser extent, as previously observed[5,6]. To quantitate the increase in kinase activity on interferon treatment, histone bands were cut out of the dried gel (using the autoradiograms as a guide) and counted in a liquid scintillation counter. There was no apparent increase in the phosphorylation of histones by enzyme fractions from two EC cell lines (PCC3/A/1 and PCC4 AzaR1) after interferon treatment, whereas L929 and 3/A/1-D-1 M extracts showed, respectively, 2.6- and 2.8-fold increases (Table 1).

In contrast to these differences in kinase induction, both undifferentiated and differentiated cells showed similarly enhanced levels of 2-5A synthetase after interferon treatment (Table 1). The level of 2-5A synthetase in different extracts was quantitated by the capacity of the poly(I) . poly(C)–Sepharose-bound enzyme fraction to synthesise 2-5A on incubation with ATP. The heat-stable (95 °C for 10 min) inhibitor of cell-free protein synthesis generated on incubation of enzyme fractions from teratocarcinoma cells showed the same spectrum of resistance and sensitivity to alkali, micrococcal nuclease, snake venom phosphodiesterase and bacterial alkaline phosphatase as the corresponding material synthesised with extracts from L929 cells or rabbit reticulocytes (data not shown, similar to those in ref. 21), thus confirming that the inhibitor synthesised was the 2'5'-linked oligoadenylic acid triphosphate.

Treatment of cells with interferon induces both antiviral and anticellular effects[24,25]. These were assayed by the resistance to vesicular stomatitus virus (VSV) infection, and by inhibition of cell multiplication. As interferon does not induce an antiviral state in EC cells[4], it was of interest to attempt to correlate the induction of 2-5A synthetase in these cells with the anticellular

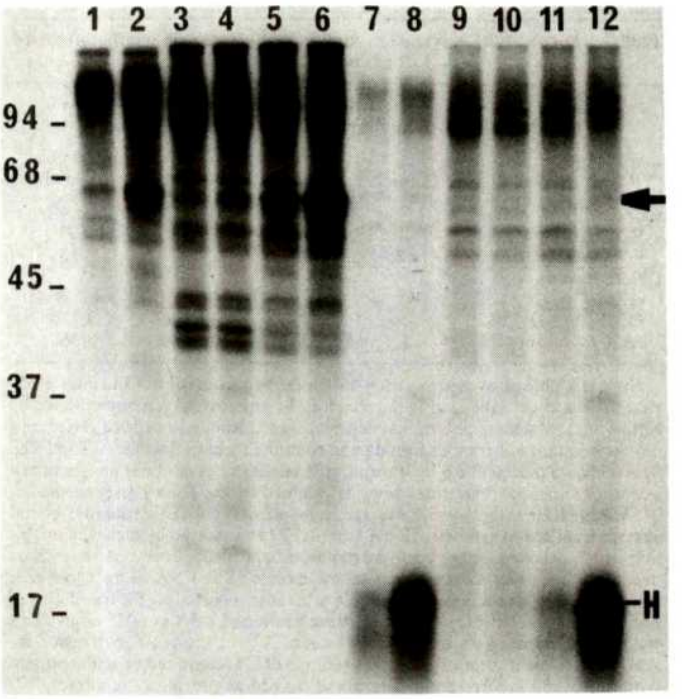


Fig. 1 Protein kinase activity of partially purified enzyme(s) fractions from control and interferon-treated cells: electrophoretic analysis on polyacrylamide (10%) slab gels containing SDS[19,20]. An autoradiograph of a stained, dried gel is shown. Chromatography on columns of poly((I) . Poly(C)–Sepharose, preparation of cell extracts and assay of the 67,000-MW polypeptide and histone kinase activities were as described previously[5,10,21]. PCC3/A/1, PCC4 AzaR1 and PCC3/A/1-D-1 M cells were grown in Dulbecco modified Eagle's medium containing 15% fetal calf serum (Flow) in a 10% CO_2 humidified atmosphere at 37 °C. L929 cells were grown in basic medium (Eagle) with Earle's salts and 10% calf serum in otherwise similar conditions. Cell extracts (5,000*g* supernatant) were from control (lanes 1, 3, 5, 7, 9, 11) and interferon-treated (250 units ml^{-1}, 17 h) (lanes 2, 4, 6, 8, 10, 12) mouse cells. Cell lines: L929, 1, 2, 7, 8; PCC3/A/1, lanes 3, 4, 9, 10; PCCS/A/1–D–1M, lanes 5, 6, 11, 12; 67,000-MW polypeptide kinase, lanes 1–6. Poly(I) . poly(C)–Sepharose-bound enzyme(s) fractions from control and interferon-treated cells were incubated for 60 min at 30 °C with 0.15 mM[γ-^{32}P]ATP (7.1 Ci $mmol^{-1}$, Radiochemical Centre) in buffer containing glycerol (10.mM HEPES, 90 mM KCl, 10 mM magnesium acetate, 7 mM 2-mercaptoethanol and 20% glycerol, v/v). Phosphorylation of histone, lanes 7–12: poly(I) . poly(C)–Sepharose-bound enzyme fractions from control and interferon-treated cells were incubated (60 min, 30 °C) with 1 mM ATP. Calf thymus histone (Type IIA, Sigma, 1 mg ml^{-1}) and [γ-^{32}P]ATP (0.15 mM as above) were added and incubation continued for 60 min. All the samples (1–12) were heated (95 °C, 10 min) in electrophoresis sample buffer containing SDS and supernatants equivalent to 0.3 mg of cell extract applied to the gel. The position of the 67,000 MW polypeptide (arrow) and histone (H) are indicated to the right of the gel. The numbers to the left give the MWs of protein markers in thousands: phosphorylase B, 94; bovine plasma albumin, 68; ovalbumin, 45; chymotrypsinogen, 37; myoglobin, 17.5. The interferon used was an electrophoretically homogenous preparation of specific activity 2×10^9 units per mg, and was prepared and provided by E. De Mayer.

effects of interferon. As expected, no resistance to VSV was induced in EC cells by interferon, whereas L929 and 3/A/1-D-1 M cells showed complete resistance to viral infection (Table 2). Similarly the interferon-mediated inhibition of cell growth in L929 and 3/A/1-D-1 M cells (58% and 42%) was not observed in EC cells, even at high concentrations of interferon (Table 2). Interferon therefore induces the 2-5A synthetase in EC cells without consequent antiviral or anticellular effects. Previous observations on the response of embryonic tissue to interferon have demonstrated a lack of cell growth inhibitory activity[26] and a low antiviral response which, however, increases with age[27].

Table 1 Levels of 2-5A synthetase and protein kinase(s) in interferon-treated and control cells

Cell type	2-5A synthetase (pmol $mg^{-1}h^{-1}$)		Histone kinase (cpm $mg^{-1}h^{-1}$)	
	Control	Interferon-treated	Control	Interferon-treated
EC cells				
PCC3/A/1	<2	225	664	697
PCC4AzaR1	<2	575	987	953
Differentiated cells				
PCC3/A/1-D-1 M	2	418	1,346	3,774
L929	8	562	1,280	3,360

Histone kinase assays were carried out on extracts from control and interferon-treated cells as described in Fig. 1 legend. Relative activities were quantitated by cutting out the bands of phosphorylated exogenous histones from dried gels using a developed autoradiogram as a guide and counting them in liquid scintillant. The figures shown represent c.p.m. incorporated into histones per hour per mg of cell extract. Protein concentrations were determined by the Folin Lowry method[22]. 2-5A synthetase activity was measured using poly(I) . poly(C)–Sepharose-bound enzyme fractions to synthesise 2-5A from ATP[6] and assayed by means of inhibition of ^{14}C-labelled amino acid incorporation in a mouse L cell-free system as described[5,6]. Translation of turnip yellow mosaic virus RNA in an L cell-free system was carried out in similar conditions to those reported for the translation of encephalomyocarditis RNA[20,23]. The concentration of 2-5A in AMP equivalents was calculated from absorbance on the basis of A_{260} of 15.6×10^3 mol^{-1} cm^{-1} for AMP. The concentration of a standard solution of 2-5A required to inhibit protein synthesis by 50% corresponded to about 0.5 nM as previously reported[6,10,21]. 2-5A synthetase activities were calculated on this basis and are given as units corresponding to 1 pmol of 2-5A synthesised per hour per mg of cell extract.

Our present results agree with these observations and clearly show that there is no correlation between the induction of 2-5A synthetase and the establishment of an antiviral state, or anticellular effects in undifferentiated EC cells. However, one cannot exclude the possibility that the induction of the 2-5A synthetase, although not conferring an antiviral state in EC cells, might nevertheless have such a role in normal tissue, in conjunction with other factors such as enhanced kinase activity. Moreover, although the 2-5A synthetase activities in EC cells and differentiated cells may be the same, the absence of the 2-5A activated nuclease would presumably counter the *in vivo* effects of enhanced synthetase activity. We therefore measured the rate of degradation of VSV mRNA into acid-insoluble material by cell extracts incubated in the presence and absence of 2-5A (data not shown, similar to Fig. 6 in ref. 28). Enhanced nuclease activity was present in all cell extracts when 2-5A was added, suggesting that the 2-5A-dependent nuclease is present in both EC and differentiated cells. Nevertheless, it is still possible that the synthesis of 2-5A or its action could be inhibited in EC cells. In addition, the levels of synthetase activity measured here probably do not accurately reflect the *in vivo* activity of the enzyme, but rather its physical presence in, perhaps, a dormant form[29]. It should also be emphasised that as yet there is no direct evidence for any role of the 2-5A synthetase and protein kinase in the establishment of the antiviral response. The induction of these enzymes by interferon, therefore, may or may not be essential for the inhibitory process in virus replication in the interferon-treated and virus-infected cell. Whatever the situation, a function of 2-5A synthetase in interferon-treated EC cells remains to be established.

Table 2 Antiviral and anticellular effects of interferon

Cell type	Antiviral effects (log_{10} pfu ml^{-1})		Anticellular effects (cell number $\times 10^5$)		
				Interferon-treated	
EC cells	Control	Interferon-treated	Control	250 U ml^{-1}	3,000 U ml^{-1}
PCC3/A/1	2.1	2.3	21.2	23.3	21.0
PCC4AzaR1	3.0	3.0	61.4	65.0	70.0
Differentiated cells					
PCC3/A/1-D-1 M	2.4	>5.0	50.4	31.0	29.4
L929	2.4	>5.0	13.9	11.8	5.8

The antiviral state of control and interferon-treated cultures was compared by challenging cells with serial twofold dilutions of VSV and determining the concentration of the virus which could kill 50% of the challenged cells after 36 h. Tests were carried out in quadruplicate, using Nunclon 96-well microplaques containing about 30,000 cells per well. The figures represent the plaque-forming units (PFU) per ml of VSV causing 50% cytotoxicity on microscopic examination. These tests were carried out on the same cell batches used for synthetase and kinase assays (Table 1). Anticellular effects were measured on cells incubated with 250 or 3,000 U ml^{-1} interferon. Cells were plated out at a density of $1–2 \times 10^5$ cells per well in Linbro (6×4) 16-mm tissue culture dishes in the presence or absence of interferon. Cell numbers were counted daily for 3 d in quadruplicate treated and control cultures. The figures shown represent cell numbers per well at day 3, when the anticellular effects of interferon were most pronounced.

As interferon interacts with EC cells without causing any antiviral or anticellular effects, a comparison between EC cells and their differentiated counterparts may allow the distinction between those interferon-induced events essential for antiviral activity, and other consequences of interferon treatment. A correlation between antiviral and anticellular effects and enhanced kinase activity is confirmed in the present study. This interferon inducibility of the kinase may, in addition, provide a useful marker for distinguishing EC cells from differentiated cells in developmental studies using teratocarcinoma[30].

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Prostaglandin D_2 controls pulmonary metastasis of malignant melanoma cells

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Selective metastasis of malignant cells in various neoplastic processes is poorly understood. Metastasis seems to be associated with inherent biochemical and biological properties of tumour cells which guarantee their survival and multiplication. These properties may include surface enzymes[1], interaction with various host defence systems and cells[2–4], combination with host

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platelets[5], or with the host endothelial cells, and adhesiveness of the metastatic cell. Fidler[2,6,7] has developed a series of malignant melanoma cells that are useful for studying tumour metastasis. By comparing the moderate metastatic parent line, $B16F_1$, to the highly metastatic line, $B16F_{10}$, it is possible to study metastasis selectively. Prostaglandins influence various cell functions, and may be involved in regulation of neoplastic processes[8-10]. We have recently reported that prostaglandin D_2 (PGD_2) formation by malignant melanoma cells *in vitro* is inversely correlated with their metastatic potential[11]. The studies we describe here were designed to determine whether treating the cells with a cyclooxygenase inhibitor, which would block conversion of arachidonic acid to prostaglandins, would affect the metastatic rate of either cell. The results suggest that PGD_2 is involved in the metastasis of these cells.

Monolayers of $B16F_1$ and $B16F_{10}$ cells which had reached confluency (in 75-cm^2 plastic tissue culture flasks, grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum) were incubated for 2 h with 20 $\mu g\ ml^{-1}$ of indomethacin, an irreversible fatty acid cyclooxygenase inhibitor[12]. Growth medium containing indomethacin was removed from the monolayers. Cells were then rinsed twice with phosphate buffer saline and then 10 ml per flask of Hank's balance salt solution without magnesium or calcium supplemented with 2% fetal calf serum was added to each flask. Cells were removed with rubber policemen and pipetted gently to achieve a single cell suspension. Cells were counted, suspended at 2.5×10^5 viable cells per ml and were distributed into plastic tubes, 2 ml per tube. Within 15–20 min of collection, cells were injected intravenously into C57BL/6J mice (Jackson Laboratories). Each mouse received 0.1 ml of cell suspension. Mice were housed in stainless steel cages and were killed 3 weeks later. Lungs were removed, fixed in 3.7% formalin solution, and the number of metastatic foci on each lung was determined using an American Optical stereo zoom microscope.

Injection of $B16F_1$ and $B16F_{10}$ malignant melanoma cells into their syngeneic host confirmed that the cells retained their differential metastatic phenotype. $B16F_{10}$ cells formed 5.1 ± 0.78 metastatic nodules per animal compared to $B16F_1$ cells which formed 1.7 ± 0.34 metastatic nodules per animal (Table 1). After treatment of the cells with indomethacin, *in vitro*, both $B16F_1$ and $B16F_{10}$ metastasis increased noticeably and there was a normalisation of metastatic differences between the two cells: $B16F_{10} = 8.9 \pm 2.8$ and $B16F_1 = 9.9 \pm 4.1$ (Table 1).

To extend these observations, the previous experiment was repeated. In this case, after cells were collected and suspended at 2.5×10^5 cells per ml in Hank's balance salt solution without magnesium or calcium, one tube received PGD_2 at a concentration that would give 2 $mg\ kg^{-1}$ PGD_2 when 0.1 ml was injected intravenously. Cells again were immediately injected into mice intravenously and 3 weeks later the number of metastatic foci per lung was determined. Results are presented in Table 2. These results confirmed again that indomethacin treatment increased the metastatic rate of the cells, particularly the less metastatic $B16F_1$ line. PGD_2 injection reversed, and decreased indomethacin-enhanced metastasis threefold. In these conditions, indomethacin-treated F_1 cells were slightly more metastatic than untreated F_{10} cells and PGD_2 injected simultaneously with both cells reduced the rate of metastasis. In these studies, the conditions were adjusted to achieve minimum levels of control metastasis so that an increase due to indomethacin treatment and any subsequent reversal by PGD_2 treatment would be more apparent. Although not shown, injection of prostaglandin $F_{2\alpha}$ and E_2 at 2 $mg\ kg^{-1}$ did not significantly affect the metastatic rate of either cell.

Prostaglandin D_2 is the principal arachidonic acid metabolite produced by $B16F_1$ and $B16F_{10}$ malignant melanoma cells[11]. We have previously demonstrated that a quantitative difference exists between the ability of $B16F_1$ and $B16F_{10}$ cells to metabolise arachidonic acid to PGD_2 and that this *in vitro* difference correlated inversely with their metastatic rate *in vivo*. Results presented here extend these observations and indicate that *in vitro* treatment of these cells with a compound that blocked conversion of arachidonic acid to prostaglandin increased the metastatic rate of the cells *in vivo*. This was particularly true for

Table 1 Pretreatment of $B16F_1$ and $B16F_{10}$ malignant melanoma cells with cyclooxygenase inhibitor normalises their metastatic potential

No. of metastatic colonies on lung surface (bilateral)			
a	*b*	*c*	*d*
F_{10} control*	F_{10} + indomethacin	F_1 control	F_1 + indomethacin
9	2	2	3
7	3	1	10
2	10	1	12
4	5	3	33
6	23	1	3
5	19	1	4
5	3	3	4
3	6		
Mean ± s.e.m.			
5.1 ± 0.78	8.9 ± 2.8	1.7 ± 0.34	9.9 ± 4.1

B16 melanoma cells were incubated for 2 h at 37 °C with growth media or 20 $\mu g\ ml^{-1}$ indomethacin. They were then rinsed with PBS and suspended at 2.5×10^5 cells ml^{-1} and 0.1 ml injected i.v. Three weeks later mice were killed, lungs were removed and the number of metastatic lesions per lung counted.

* Using the Fisher's Exact Test, at the $P \leq 0.05$ level: $a < b$; $a > c$; $c < d$.

Table 2 Prostaglandin D_2 reduction of the metastatic potential of B16 malignant melanoma cells

No. of metastatic colonies on lung surface (bilateral)					
*a**	*b*	*c*	*d*	*e*	*f*
F_{10} control	F_{10} + INDO	F_{10} + INDO + PGD_2	F_1 Control	F_1 + INDO	F_1 + INDO + PGD_2
1	11	2	0		1
1	2	1	0	2	2
2	2	0	0	10	0
1	4	2	0	20	4
0	2	1	0	16	0
3	2	0	0	9	2
2	2	1	3	6	10
10	4	1	1	2	2
3	3	0	1	20	15
1	3	2	1	3	
Mean ± s.e.m.					
2.4 ± 0.88	3.5 ± 0.85	1.0 ± 0.25	0.6 ± 0.30	8.8 ± 2.3	3.6 ± 1.6

$B16F_1$ and $B16F_{10}$ cells were incubated for 2 h with 20 $\mu g\ ml^{-1}$ indomethacin (INDO), were then rinsed with PBS and resuspended at 2.5×10^5 cells ml^{-1}. To one half of the treated cells PGD_2 was added to give 2 $mg\ kg^{-1}$ when 0.1 ml of the cell suspension was injected i.v. Lungs were collected 3 weeks later, were fixed with 3.7% formalin and metastatic foci were counted using a dissecting microscope.

* Using the Fisher's Exact Test, at the $P \leq 0.05$ level: $a > c$ but not b; $b > c$; $a > d$; $d < e$ and f and $e > f$.

the $B16F_1$ cells that were less metastatic but produced the most PGD_2. Indomethacin treated cells were also co-injected with PGD_2. If PGD_2 was controlling the metastatic rate of the malignant melanoma cells, exogenous reconstitution of their PGD_2 levels should reverse the effect of indomethacin. This was found to be the case. Cells that were made more metastatic by indomethacin treatment reverted to a less metastatic form on co-administration with PGD_2.

One mechanism proposed for B16 cellular metastasis is that platelets adhere to the surface of malignant cells, causing emboli which then plug or attach to the endothelial surface in capillary beds[5].

Prostaglandin D_2 in sufficient amounts inhibits platelet aggregation in several species[13,14], and we have verified this effect in C57B1/6J mice. Our results suggest that PGD_2 is directly involved in prevention of pulmonary metastasis, possibly through its influence on the formation of platelet–tumour emboli. Other effects of PGD_2 on cell–cell interactions, cellular membranes and host defence systems may also be involved.

Indomethacin neutralised the ability of these cells to make PGD_2 and increased their metastatic rate. Exogenous restoration of PGD_2 reversed the effect of indomethacin. The consolidated data from *in vitro* experiments[11] and these *in vivo* experiments are consistent with a role of PGD_2 in the inhibition of platelet–tumour emboli. These results suggest that PGD_2 is involved directly in control of metastasis of these malignant melanoma cells. It is not presumed, however, that this is the only mechanism controlling the metastatic rate of these cells. Other biochemical and biological properties of the cells may be intimately involved in the metastatic rate. Results do suggest, however, that PGD_2 is one natural, cellular component controlling pulmonary metastasis of malignant melanoma cells.

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Naloxone-reversible effect of opioids on pinocytosis in *Amoeba proteus*

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A characteristic feature of induced pinocytosis in *Amoeba proteus* is the formation of broad channels by invagination of the cell membrane. This process, which requires Ca^{2+}, occurs in response to depolarising cations[1–4]. High Ca^{2+} levels reduce pinocytosis induced by cations such as Na^+ and $Tris^+$, whereas pinocytosis induced by K^+ is less affected by Ca^{2+} (ref. 4). Agents which interfere with the calcium metabolism of the amoeba will therefore either stimulate or inhibit pinocytosis induced by Na^+ (ref. 5). Among the agents which are supposed to reduce Ca^{2+} influx across cell membranes or otherwise decrease cellular availability of Ca^{2+} are the opiates and opioid peptides[6–9], high doses of which have been reported to affect the amoeba[10]. Accordingly, Met-enkephalin, morphine and codeine potentiate the inhibition of pinocytosis caused by Ca^{2+}-binding agents and reverse the calcium blockade of pinocytosis mediated by caffeine[11]. In this report we show that pinocytosis induced by Na^+ or $Tris^+$ is suppressed by β-endorphin, Met-enkephalin and morphine. These effects were abolished or diminished by an opiate receptor antagonist, (−)naloxone, by increasing the Na^+ concentration, or by addition of Ca^{2+}.

We used *Tetrahymena*-fed *Amoeba proteus* which were cultured in a modified Pringsheim medium[12] or in Prescott–James solution[13]. The results were similar irrespective of culture medium. Pinocytosis was induced by removal of the medium and addition of the inducing solution (100 mM NaCl at *p*H 6.0, 90 mM NaCl plus 10 mM Tris-HCl at *p*H 7.0, 100 mM Tris-HCl at *p*H 7.0 or culture media containing 0.1 mg ml^{-1} cytochrome *c*, Trasylol or lysozyme). Pinocytosis activity was determined by counting the number of pinocytotic channels using phase-contrast microscopy. Each amoeba was inspected for 30 s and the channels in 40 amoebae were counted during the first 20 min of the pinocytosis cycle. The mean number of channels per amoeba was taken as an index of pinocytosis activity. Further details of the methods for culture of cells and assay of pinocytosis activity are described elsewhere[3,14].

Morphine (4–40 nM), Met-enkephalin (0.2–2 nM) or β-endorphin (0.1–20 nM) (which we will describe collectively as opioids, to include both opiates and opioid peptides) decreased Na^+-induced pinocytosis by up to 75%. Only low doses of the three opioids were effective and so the relationship between the inhibitory effect of opioids and pinocytosis gave rise to U-shaped dose–response curves. Also, pinocytosis induced by cytochrome *c*, Trasylol or lysozyme was inhibited by the opioids (Table 1). These cationic proteins give rise to pinocytosis which resembles that induced by Na^+ with respect to Ca^{2+} requirements[3,4]. Pinocytosis induced by KCl, on the other hand, was not significantly influenced by the opioids. Low concentrations of (−)naloxone reversed the effects of the opioids whereas the same concentrations of (+)naloxone were not antagonistic. (+)Naloxone possessed about 1/10,000th the activity of (−)naloxone (Fig. 1). A similar low potency of (+)naloxone in three mammalian assay systems has been reported[15].

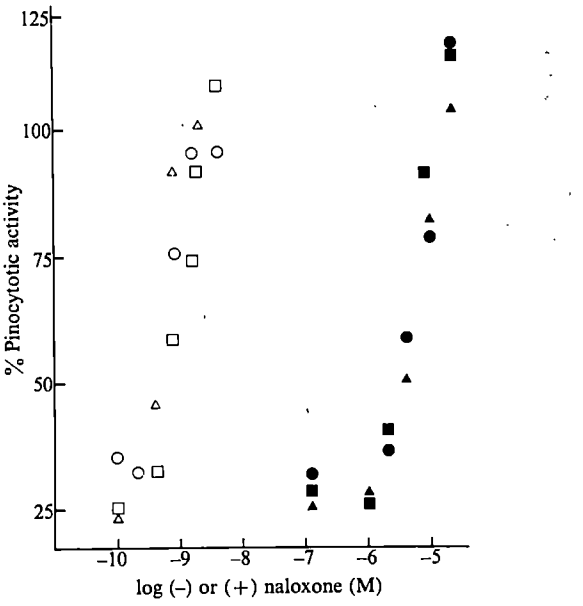


Fig. 1 Reversal by (−)naloxone (open symbols) and (+)naloxone (filled symbols) of the inhibition of pinocytosis due to 8 nM morphine (circles), 0.3 nM β-endorphin (squares) and 0.3 nM Met-enkephalin (triangles). Pinocytosis was induced by 100 mM NaCl, *p*H 6.0. The pinocytotic activities are given as per cent of the controls. The drugs were added to the inducing salt solution and the experiments were carried out at 23°C. Each experimental point represents a mean value of pinocytosis activity obtained from 40 amoebae during the first 20 min after induction. Note that the first part of the ordinate is deleted.

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Table 1 Effect of opioids and naloxone on pinocytosis induced by NaCl (*a*) and cationic proteins (*b*)

a	NaCl 100 mM, *p*H 6.0	Plus 2 nM (−)naloxone	Plus 2 nM (+)naloxone	Plus 20 μM (+)naloxone
Control	100*	95±1.5 (7)	100±4.0 (7)	99±2.2 (7)
Morphine 8 nM	34±2.8 (7)	97±3.2 (7)	35±3.7 (7)	77±10.2 (7)
β-Endorphin 0.3 nM	39±5.1 (7)	90±3.9 (7)	33±3.8 (7)	75±10.4 (7)
Met-enkephalin 0.3 nM	28±2.2 (7)	95±6.6 (7)	25±2.0 (7)	

b	Cytochrome *c* 0.1 mg ml^{-1}	Plus 2 nM (−)naloxone	Lysozyme 0.08 mg ml^{-1}	Plus 2 nM (−)naloxone	Trasylol 0.1 mg ml^{-1}	Plus 2 nM (−)naloxone
Control	100*	101±2.9 (3)	100*	97±8.1 (3)	100*	102±3.8 (3)
Morphine 8 nM	55±4.5 (5)	109±5.8 (5)	44±3.6 (5)	93±3.2 (5)	56±6.3 (5)	107±11.3 (5)
β-Endorphin 0.3 nM	52±6.1 (4)	98±2.4 (4)	49±4.4 (4)	95±6.0 (4)	53±4.4 (5)	97±1.5 (5)

(+)Naloxone was synthesised[15] by A. E. Jacobson. Cytochrome *c* (type VI, Sigma) and Trasylol (Bayer) were dissolved in Prescott–James solution, and lysozyme HCl (Miles-Seravac) was added to the ordinary culture medium—Pringsheim solution.
* Pinocytosis intensity of controls are rated as 100%. Values are ±s.e.m. of the number of experiments given in parentheses.

The sensitivity of the amoeba to the opioids increased when Na^+ was replaced by the protonated form of Tris ($Tris^+$). This is shown in Fig. 2*a*, where substitution of $Tris^+$ for Na^+ increased the sensitivity to morphine about 10-fold. (−)Naloxone (0.6–2 nM) antagonised the effect of morphine on both Na^+- and $Tris^+$-induced pinocytosis (Fig. 2*b*). The results of this and similar experiments suggest that the potency of the antagonist against morphine or Met-enkephalin (Fig. 2*d*) is increased in the presence of Na^+. Compared with morphine, Met-enkephalin and β-endorphin were less influenced by Na^+ (Figs 2*c*, 3*c*).

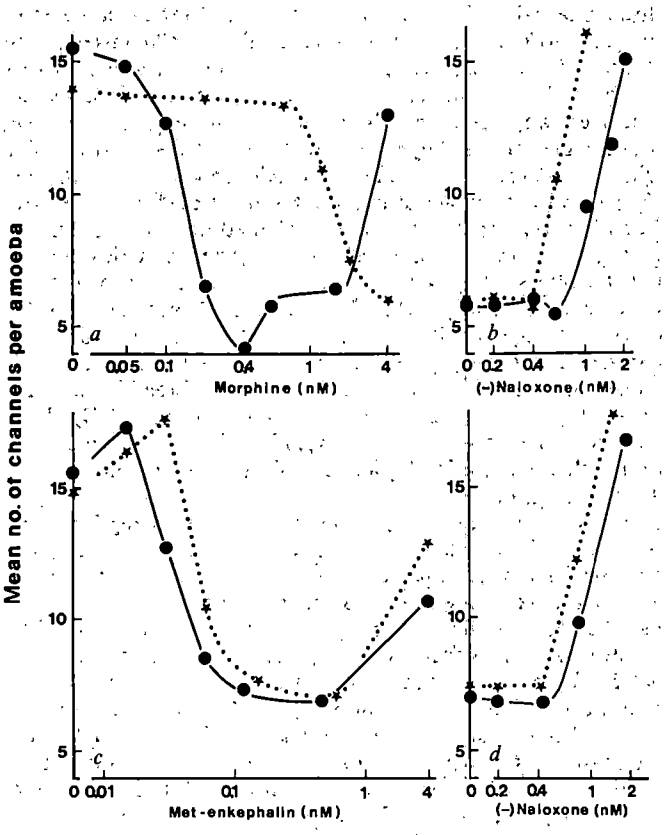


Fig. 2 The effect of morphine and Met-enkephalin on pinocytosis and its reversal by (−)naloxone. Pinocytosis was induced by 100 mM Tris-HCl (●) or 90 mM NaCl plus 10 mM Tris-HCl at *p*H 7.0 (Na^+-induced pinocytosis, ★). The conditions for the experiments were as described in Fig. 1 legend. *a*, The inhibitory effect of morphine chloride (ACO, Solna) on pinocytosis-induced by $Tris^+$(●) or Na^+(★). *b*, (−)Naloxone-HCl reverses the effect of 0.6 nM (●) and 4 nM (★) morphine on pinocytosis induced by Tris and sodium, respectively. The cells were from the cell culture used in the experiments of (*a*). *c*, The effect of Met-enkephalin on pinocytosis induced by $Tris^+$ (●) or Na^+ (★). *d*, Inhibition of pinocytosis by 0.12 nM (●) and 0.15 nM (★) Met-enkephalin (Beckman) is reversed by (−)naloxone. The same cell culture as (*c*).

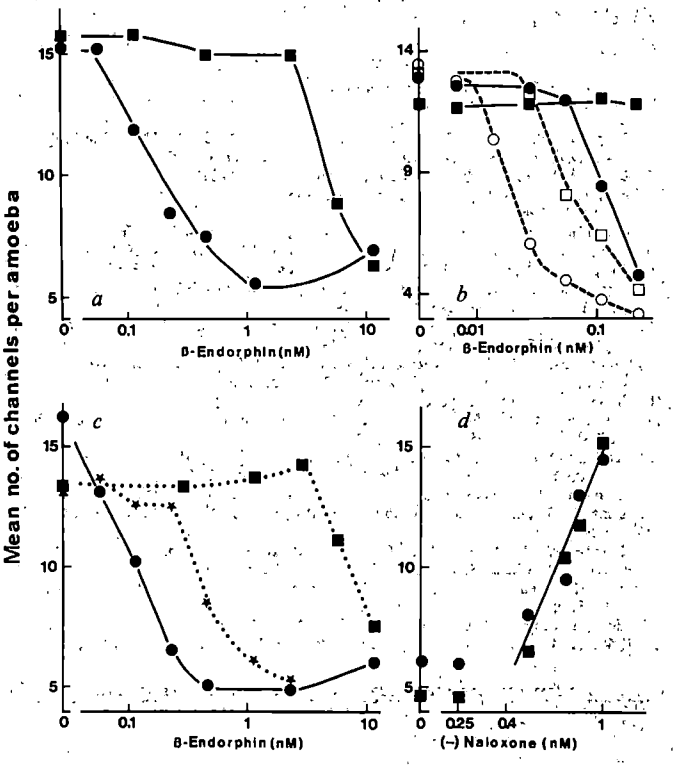


Fig. 3 The effect of Ca^{2+}, verapamil and (−)naloxone on inhibition of pinocytosis by β-endorphin. The inducing salt solutions were as outlined in Fig. 2 legend. *a*, Dose–response curves of β-endorphin (Peninsula Laboratories) on pinocytosis induced by 100 mM Tris-HCl at *p*H 7.0 (●) and by $Tris^+$ plus 10 μM $CaCl_2$ (■). *b*, Inhibition of pinocytosis by β-endorphin is potentiated by verapamil. Pinocytosis was induced by $Tris^+$ (circles) or $Tris^+$ plus 10 μM $CaCl_2$ (squares). Open symbols denote presence of 8×10^{-7} M verapamil. *c*, Comparison between the inhibitory effect of β-endorphin on pinocytosis induced by $Tris^+$ (●), Na^+ (★) or Na^+ plus 10 μM $CaCl_2$ (■). *d*, Reversal of the inhibitory effect of 10 nM β-endorphin by (−)naloxone. Pinocytosis was induced by $Tris^+$ (●) or $Tris^+$ plus 10 μM $CaCl_2$ (■).

An even more significant ionic influence was exerted by Ca^{2+}. In the presence of 10 μM $CaCl_2$ the inhibitory potency of all three opioids was decreased by approximately 90%. This is shown for β-endorphin in Fig. 3*a*. On the other hand, verapamil, which inhibits Ca^{2+} influx in many cells[16], potentiated the response to the opioids and abolished the effect of Ca^{2+} (Fig. 3*b*). Unlike Na^+, Ca^{2+} did not affect the sensitivity to (−)naloxone (Fig. 3*d*) but decreased that to the opioids even when Na^+ was the inducing cation (Fig. 3*c*).

The low concentrations of opioids which affect pinocytosis in *A. proteus* and the enantiomeric stereospecificity of the antagonistic effect of (−)naloxone demonstrated here strongly suggest that these drugs interact at a receptor level. The finding

that the response to opioids in the amoeba is sensitive to Na^+, may reflect conformational changes of the receptor molecule, similar to those which reduce binding of agonists to opiate receptors in nervous tissue[17,18]. Furthermore, the difference in Na^+ sensitivity between Met-enkephalin and morphine in binding to mammalian opiate receptors[19] and inhibition of pinocytosis is similar. The opioids may alter the pinocytotic activity either by reducing the intracellular Ca^{2+} or by decreasing the sensitivity to this ion. Their mode of action may, in fact, be similar to that in synaptosomes, where they are reported to inhibit Ca^{2+} influx[8,20]. However, a physiological function for the opiate receptors in, for example, calcium regulation is difficult to conceive unless ligands for them are discovered in the amoeba itself or in its ecosystem.

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Adult rat brain astrocytes support survival of both NGF-dependent and NGF-insensitive neurones

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The hypothesis that trophic substances are involved in the normal development and maintenance of the autonomic nervous system has gained much support from studies on the function of nerve growth factor (NGF). *In vitro* at least, both avian and mammalian sensory and sympathetic neurones have a requirement for NGF[1], or for similar, as yet uncharacterised, molecules from homologous non-neuronal cells[2], glial cells[3], glioma cells[4] or homogenates of certain tissues[5]. Although molecules other than NGF can apparently support the *in vitro* survival of apparently NGF-sensitive neurones[4,5], there has been no definitive evidence—excluding preliminary results with parasympathetic neurones of the ciliary ganglion[6–8]—that other specific trophic factors are essential for neurones insensitive to NGF. I report here that adult rat brain astrocytes can promote the *in vitro* survival of not only NGF-dependent neurones, such as rat embryo superior cervical ganglion (SCG) cells, but also NGF-insensitive neurones such as the sensory neurones of the rat or chick embryo nodose ganglion (NG). The support of the NGF-dependent neurones is mediated by astrocyte-derived NGF, whereas the survival of NGF-insensitive neurones is brought about by a diffusible factor quite distinct from NGF, but as yet uncharacterised. Evidence is also presented which indicates that the astrocytes can support two distinct populations of neurones in the chick dorsal root ganglion (DRG). It is suggested that these populations represent respectively the previously described small, late-developing, medio-dorsal, NGF-dependent neurones and the early developing, large, ventro-lateral, NGF-insensitive neurones[9].

Astrocytic glial cells, designated RG18, derived from normal adult rat brain after kainate lesioning of the corpus striatum were maintained as monolayer cultures in 35-mm plastic (Falcon) tissue culture dishes, as previously described[10]. Experimental RG18 were seeded (5×10^3 cells per dish) to give almost confluent monolayers at the start of neuronal–astrocyte co-culture. Rat SCG and NG were from 17–19-d-old embryos, and chick DRG and NG were from 8–9-d-old embryos. Rat ganglia were dissected free of connective tissue, washed twice with Eagle's minimal essential medium (MEM), teased into small chunks and digested with 0.25% trypsin in MEM for 30 min at 37 °C. Chick embryo ganglia required less vigorous trypsinisation—10 min with 0.1%. Trypsinised ganglia were washed three times with MEM, and dissociated into single-cell suspensions by trituration through a finely drawn siliconised glass Pasteur pipette. Cell suspensions were filtered through 45-μm nylon mesh and counted in a haemocytometer. Dissociated neurones were seeded either directly on to the astrocyte monolayer or the astrocytes were first covered, 3–4 h previously, with 1.0 ml of reconstituted rat tail collagen gel[11]. The collagen prevents direct contact of neurones and astrocytes but permits free diffusion between them and the medium. The co-culture growth medium (2 ml per dish) was as previously described[12] with the omission of NGF. Control cultures of all neuronal types were grown on collagen gel (1 ml) coated 35-mm dishes in the presence (5 ng per dish) or absence of NGF purified as before[4]. Parallel experiments were carried out in which anti-NGF antiserum (sheep or rabbit antiserum) was included in amounts of 5–100 μg ml^{-1}. Cultures were maintained in a 5% CO_2: 95% air, humidified atmosphere at 36.5 °C. Growth medium was replenished every third day and co-cultures were maintained for 3 weeks or more.

As shown in Fig. 1, neurones from all the ganglia tested survived and extended long neurites in co-culture conditions. Very few, if any, neurones survived in control conditions, that is, in the absence of either glial cells or exogenous NGF. A modified Holmes silver stain[13] procedure was routinely used to identify neuronal processes (Fig. 1*a–c*), although the large flattened astrocytes are readily distinguished from any neurones even without staining. The survival of SCG and DRG neurones in co-culture could be the result of NGF secretion by the astrocytes. However, this would not explain the survival of nodose ganglion neurones which are insensitive to NGF both in the intact ganglion *in vivo* or *in vitro* (R. Levi-Montalcini, personal communication) and in dissociated culture (R. M. L., unpublished results). Further experiments verified that NGF or an immunologically cross-reactive molecule was being released by the RG18 cells and mediated the survival of the SCG neurones and some of the DRG neurones. Anti-NGF antiserum from two different sources completely abolished the ability of RG18 astrocytes to support the growth of SCG neurones, but only diminished the survival of DRG neurones (Table 1). Increasing the amount of antiserum beyond 5 μg per dish had no further effect on the remaining population of DRG neurones. This clearly suggests that the astrocytes do more than simply support the NGF-sensitive neurones. Nodose ganglion neurones from either chick or rat embryo which survived in

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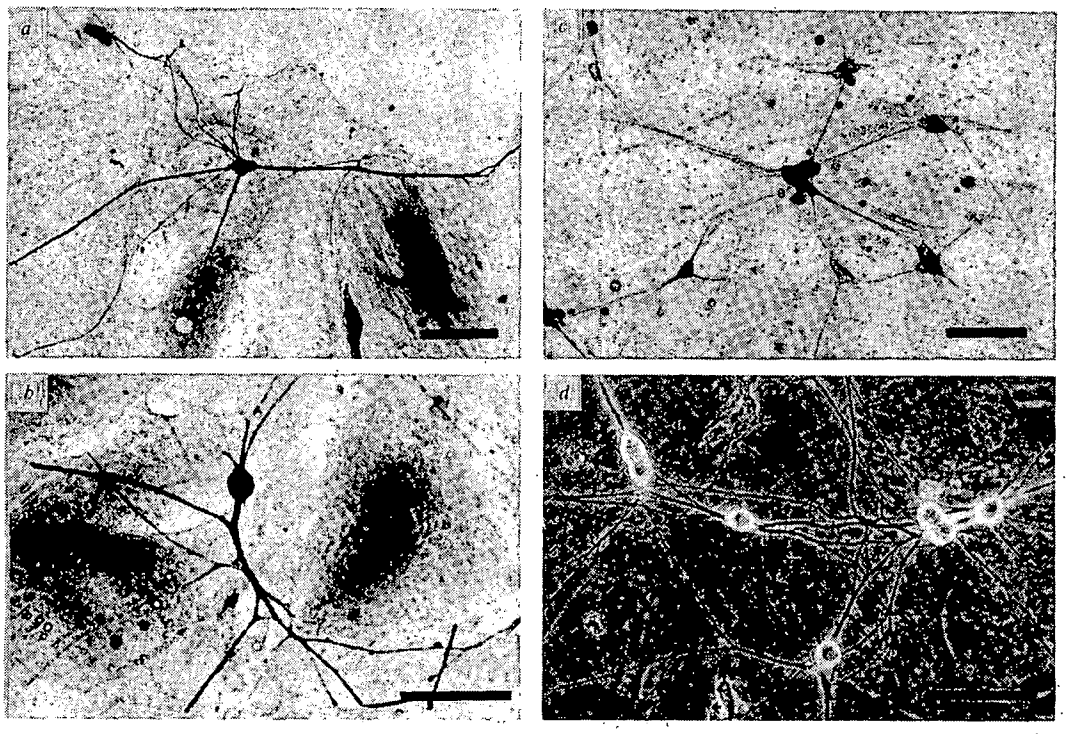


Fig. 1 Dissociated NGF-dependent (SCG, DRG) and NGF-insensitive (NG) neurones surviving in direct contact with adult rat brain astrocytes in the absence of exogenous NGF. Cultures were established as described in the text. *a*, Single rat SCG neurone straddling several RG18 cells, silver stained after 6 d in culture. *b*, Single chick embryo DRG neurone between nuclei of two RG18 cells, silver stained after 6 d *in vitro*. *c*, Network of rat embryo NG neurones on top of astrocyte monolayer, silver stained after 10 d in culture. *d*, Chick embryo NG neurones as seen by phase contrast, unstained preparation after 2 weeks in culture. Calibration bar, 100 μm. Similar observations are obtained when collagen gel is used, but the neuronal fibres extend in three dimensions, making photographic representation difficult.

co-culture with the RG18 cells were completely unaffected by NGF antiserum, as might be anticipated from their insensitivity to NGF *per se*.

To ascertain that the effect of the astrocytes on neuronal survival was not simply the result of a contact phenomenon, co-cultures were established, as described earlier, in which collagen gel was interspaced between the glial monolayer and the neurones seeded on top. Neuronal survival was generally greater when collagen was used (Table 1) than when neurones and glial cells were in direct contact. The gel has no obvious effect on the growth of either cell type.

The number of neurones seeded per dish in these experiments (Table 1) may seem low relative to other studies. The low seeding was initially the result of low yields of nodose ganglion cells after dissociation, but it was later found that low seedings gave more reproducible and more easily quantitated determinations of neuronal survival. However, the low values do not represent a limitation of the astrocytes to support only a few cells, as an increase in the number of neurones seeded gives a proportional increase in neuronal survival (Table 2). The capacity of the astrocyte cultures in this respect has not been fully determined.

Table 1 Influence of adult rat brain astrocytes on the *in vitro* survival of sensory and sympathetic neurones

Growth surface for dissociated neurones	Exogenous NGF (ng per dish)	Anti-NGF antiserum (μg per dish)	Neuronal survival (per 35-mm Petri dish) Rat		Chick	
			SCG	NG	DRG	NG
Collagen (1 ml gel)	—	—	2±1	11±2	13±3	10±5
Collagen	5	—	270±27	6±1	208±42	12±3
Collagen	5	100*	2±1	10±4	14±1	10±5
Astrocytes	—	—	341±5	108±13	390±16	416±67
Astrocytes+collagen	—	—	348±25	152±4	818±95	307±20
Astrocytes+collagen	—	5*	22±4	ND	246±23	ND
Astrocytes+collagen	—	50*	18±17	ND	198±19	ND
Astrocytes+collagen	—	100*	16±4	141±8	213±20	310±18
Astrocytes+collagen	—	100†	10±5	163±13	259±33	288±24
Astrocytes+collagen	5	—	813±68	153±21	1,183±93	313±40

Cultures were seeded with either 5,000 superior cervical (SCG) or dorsal root ganglion (DRG) cells or 1,000 nodose ganglion (NG) cells per 35-mm dish, prepared as described in the text. Neuronal survival was determined after 6 d in culture, by counting in the phase contrast microscope all neurones with long processes, scanned for in 25% of the culture dish surface. In this typical experiment values represent the mean ± s.e.m. of triplicate dishes, evaluated by two independent counters. NGF was purified and assayed for maximal activity as before[4]. Sheep anti-NGF antiserum at 100 ng ml^{-1} was sufficient to block the activity of 1 ng ml^{-1} NGF. Rabbit anti-NGF antiserum was similarly active, and both antisera were obtained with 2.5S mouse salivary gland NGF. Astrocytes were seeded 1 week before the experiment and coated with collagen gel 3–4 h before neurones were seeded. Not more than 3 h elapsed between the removal of ganglia and the seeding of neurones. Growth medium—MEM, KCl, glucose, uridine, fluorodeoxyuridine and human placental serum[12]—was prepared freshly at each medium change. Human placental serum was obtained from a local hospital and inactivated 30 min at 56 °C before use. The presence or absence of fluorodeoxyuridine did not affect the results: ND, Not determined.

* Sheep antiserum; † rabbit antiserum.

Table 2 Increase in survival of nodose ganglion (NG) neurones with increase in number seeded

Growth surface	Ganglion cells seeded ($\times 10^{-3}$)	Neuronal survival (per 35-mm dish) Rat NG	Chick NG
Collagen	5	8	24
Collagen + NGF (5 ng)	5	31	72
Astrocytes + collagen	5	728	664
Astrocytes + collagen	10	928	736
Astrocytes + collagen	20	1,912	1,624
Astrocytes + collagen	30	4,328	3,104
Astrocytes + collagen	40	5,504	5,752

Cultures were established as described in the text and Table 1 legend. Neuronal survival was determined after 5 d in culture. Values are for single samples only.

The present findings are consistent with the notion that there are molecules analogous to NGF which are essential for the growth and survival of NGF-insensitive neurones. It also supports the role of glial cells in glial–neuronal interactions, and confirms reports that cells of glial origin (glioma[14], glial[15]) are capable of synthesising functionally active NGF. It has previously been shown[9] that not all cells of the DRG are sensitive to NGF and at least two distinct populations of neurones have been identified in the ganglion by this and other criteria. The present results indicate that astrocytes are capable of promoting survival of not only the NGF-dependent neurones (the small mediodorsal cells) but also some NGF-insensitive neurones. This is borne out by the observation that anti-NGF antiserum can diminish but is unable to abolish the ability of astrocytes to support DRG neurones in co-culture (Table 1). The developmental similarity between the large neurones of the nodose ganglion and the large, ventro-lateral, NGF-insensitive neurones of the DRG suggests that these latter neurones survive in astrocyte co-culture and that it is their survival which is unaffected by anti-NGF antiserum. Verification of this latter point by biochemical and immunohistological analyses of the surviving cells in different conditions is in progress.

It will be interesting to determine through cell selection procedures whether a single astrocyte from the RG18 cultures is capable of synthesising more than one neuronal growth factor, or whether the ability to synthesise NGF and the ability to support the survival of NGF-insensitive neurones reside in different glial cell types. It will also be interesting to establish if there is any connection between the results of this study and previous reports of non-NGF neuronal growth-promoting activity in glioma cultures[4] or homogenates of certain tissues[5].

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Onset of neural function in the lateral line

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The development of the nervous system includes the formation of specific neuronal connections. Some insight into the mechanisms by which these connections are made may be obtained by determining the order in which the elements of a developing system begin to function and examining any changes that may occur in the system shortly after it begins functioning. I have investigated the lateral line of the South African clawed frog, *Xenopus laevis* and I have classified the afferent system into three elements. First, the transduction mechanism, which includes all components that couple the water vibrations to the release of transmitter from the hair cell. Second, the afferent synapse, at which there is transmitter release and subsequent postsynaptic conductance changes. And third, the afferent axon. I report here experiments suggesting that these elements become functional in the following order: first, the axon can conduct action potentials; second, transmission is established; third, the transduction mechanism can modulate transmitter release. This same sequence has been reported in a system related to the lateral line, the auditory pathway of mammals[1].

The peripheral lateral line basically consists of many clusters (neuromasts) of hair cells and a nerve connecting these sensory cells to the brain[2]. Two afferent nerve fibres innervate a neuromast; each connects with half of the hair cells, all of which are orientated in the same direction and opposite to those synapsing on to the other fibre. The hairs project into the water environment and sense aqueous vibrations. In addition, there are efferent fibres which make inhibitory synapses on to the hair cells[3]. At the earliest developmental time (26 days stage 34 (ref. 4)) when electrical activity in the lateral-line nerves of *Xenopus* tadpoles has been examined it has been essentially adult in character[5], indicating that the developmental events mentioned above are completed by that time. The experiments reported here were carried out on *Xenopus* tadpoles between the ages of 2 days 8 h (NF 38) and 6 days (NF 48) after fertilisation of the egg (timing accurate to within 2 h). The animal was immersed in saline (3 mM KCl, 10 mM $CaCl_2$, 125 mM NaCl, 5 mM Tris-HCl, *p*H 7.3–7.4), anaesthetised by chilling to 1 °C,

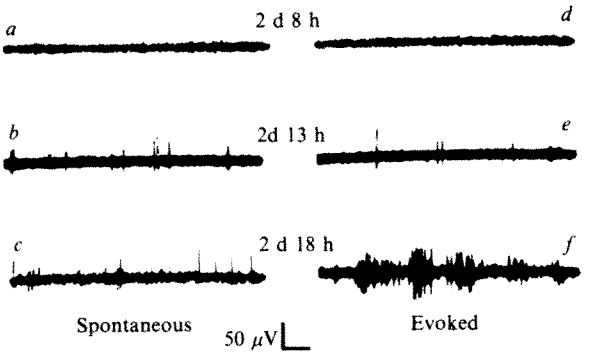


Fig. 1 Electrical activity recorded from lateral-line nerve at three ages. *a–c*, No stimulus: *d–f*, gross stimulation. *a*, *d*, 2 d 8-h-old animal. *b*, *e*, 2 d 13-h-old animal. *c*, *f*, 2 d 18-h-old animal. Records from three different animals. Of five animals recorded from at 2 d 13 h exhibiting spontaneous activity, none had evoked activity. Of five animals recorded from at 2 d 18 h, all had both spontaneous and evoked activity. Horizontal scale, *a*, *c*, *d*, *f*, 0.1 s; *b*, 0.5 s; *e*, 0.2 s.

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immobilised, the spinal cord transected and the brain removed. Nomarski optics were used to visualise the lateral-line nerve, the neuromasts and individual hairs (kinocilia). Removing a window of skin allowed a recording suction electrode to be applied to the post-orbital lateral-line nerve, either to its cut end or *en passant*, near its entry to the brain. Electrical activity in the nerve was recorded using standard electrophysiological techniques. Three modes of stimulation were used: (1) gross stimulation, a gentle rocking of the preparation table which caused the saline to flow back and forth across the tadpole, exciting many neuromasts; (2) a mechanical stimulation applied to single neuromasts, as described below; and (3) direct electrical stimulation of the nerve by passing current pulses through a stimulating suction electrode. All experiments were done at room temperature (20–22 °C).

The onset of sensory transmission by the afferent nerve was determined by recording from animals of different ages (Fig. 1). At 2 days 8 h, neither spontaneous nor evoked activity was seen, even though the nerve was capable of conducting impulses in response to electrical stimulation (Fig. 2).

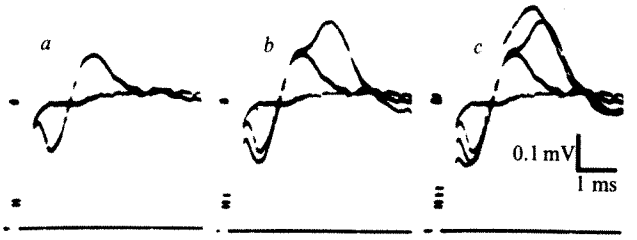


Fig. 2 Direct electrical stimulation of lateral-line nerve in 2 d 8-h-old animal with four current pulses, one every 15 s, delivered by a stimulating suction electrode. Top traces, evoked compound action potentials. Bottom traces, stimulating pulses. *a*, First two trials, superimposed, *b*, first three trials; *c*, all four trials. The fast and slow components, most easily seen in *b*, may correspond to afferent and efferent fibres, respectively.

The appearance of spontaneous activity at $2\frac{1}{2}$ days (Fig. 1*b*, *e*) suggested that transmission begins at that time. The chemical sensitivity of the synapse offers a method of determining the origin of the spontaneous activity and testing this point. Chemicals suspected of being the afferent transmitter, L-glutamate[6] (L-Glu) and L-γ-aminobutyric acid[7] (GABA), were introduced into the saline to see if either would diffuse to the junction and stop activity by desensitising the postsynaptic receptors[8,9]. The efferent synapse on to the hair cell is likely to be cholinergic[10] and would not be affected by these substances. Exposure to 1 mM L-Glu reversibly abolished spontaneous and evoked activity in animals old enough to exhibit evoked activity (Fig. 3). Spontaneous activity in younger animals was similarly eliminated. The activity usually increased shortly after introduction of L-Glu and only after several minutes fell to zero (data not shown). This pattern of activity, characteristic of desensitisation[6], was not seen when equal concentrations of GABA or a control, D-Glu, were substituted for L-Glu. The diffusion path is not known but may include passage through holes made in the skin during dissection. These results not only suggest that the afferent transmitter is L-Glu, but also indicate that the spontaneous activity arises at the junction. This interpretation is consistent with the proposal of Harris and Flock[2] that the spontaneous activity in adult *Xenopus* lateral line is caused by transmitter release and not a pacemaker potential. It remains to be determined whether the final component of transmission is the release of transmitter, the apposition of the pre- and postsynaptic membranes, or the appearance of a sufficient postsynaptic response.

At 2 days 18 h, gross stimulation evoked a vigorous response (Fig. 1*f*) in contrast to its apparent lack of effect at 2 days 13 h (Fig. 1*e*). The onset of sensory function occurs, therefore, at roughly $2\frac{3}{4}$ days. As gross stimulation does not affect synaptic transmission until this time, I conclude that the transduction mechanism is the last of the three afferent elements to function.

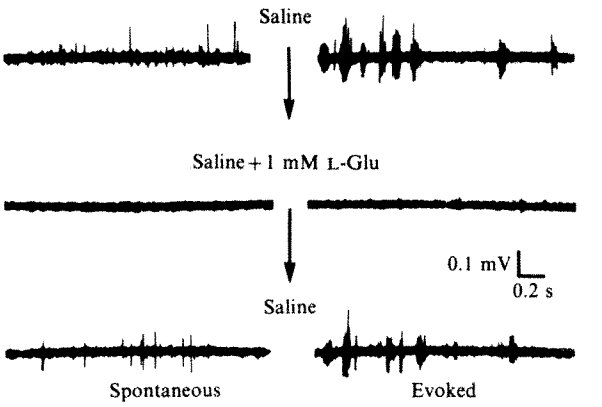


Fig. 3 Glutamate sensitivity of the lateral line in 3 d 15-h-old animal. Left records, no stimulus. Right records, gross stimulation. Top, before addition of L-Glu. Middle, 7 min, after 1 mM L-Glu (*p*H 7.4) introduced to the bath. Bottom, 9 min after wash and return to normal saline. The L-Glu concentration is comparable to that needed to desensitise Mormyrid lateral line[6] and may be necessary due to the diffusion barrier surrounding the synapse. Four out of five animals tested before the age of 3 d 10 h showed the response. After that age the system showed decreasing sensitivity to L-Glu, possibly because the diffusion path becomes progressively more impermeable with age.

Developmental changes in the sensory response of the system were studied by means of a mechanical stimulator which provided a one-dimensional controlled movement of 2–20 μm in response to an applied voltage. The sensitivity of single neuromasts was investigated by orientating the stimulator in the direction of maximum sensitivity of the neuromast[5], placing it in contact with the hairs ~10 μm above the skin surface, and vibrating it to evoke activity from that neuromast. In examination of 50 neuromasts from 18 animals, both units were often seen to respond, one to each phase of the stimulus. The sensitivity of the system increased substantially between 2 days 18 h and 6 days. For example, an equivalent response to 10 cycles of 100 Hz sinusoidal stimulation was elicited at 6 days by a stimulus whose amplitude was only 20% of that required at $2\frac{3}{4}$ days. At the onset of sensory function the system was not able to follow a 200-Hz stimulus, whereas at 6 days, it followed frequencies greater than 350 Hz. The average kinocilium length at $2\frac{3}{4}$ days is ~50% what it is at 6 days.

It seems that the nerve is the first element to function, capable of conducting action potentials before 2 days 8 h after fertilisation. Second, transmission begins at approximately $2\frac{1}{2}$ days. Third, at $2\frac{3}{4}$ days, the transduction mechanism operates, and sensory function begins, approximately 1 day after responses have been found to dimming the illumination of the pineal eye[11], and 1 day before the first tectal activity evoked by ocular stimulation with light flashes[12]. The sensitivity of the peripheral lateral line increases greatly during the next 4 days. The development of the efferent system has yet to be studied. The efferent axons may be functional before 2 days 8 h. The onset of synaptic inhibition of the hair cells has not yet been determined.

The fact that transmission begins before the transduction mechanism is established implies that this connection is formed without benefit of sensory information. In addition, the first few hours activity carried by the afferent fibres seems to be only spontaneous and unmodulated. It will be interesting to ascertain whether these first connections are altered as the animal develops further.

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Equivalence of Ca^{2+} and Sr^{2+} in transmitter release from K^+-depolarised nerve terminals

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Depolarisation–secretion coupling at presynaptic nerve terminals is thought to be mediated by the influx of calcium ions from the extracellular fluid through voltage-sensitive channels in the nerve terminal membrane[1]. In physiological conditions, the depolarisation is accomplished when the nerve action potential invades the nerve-ending. At the neuromuscular junction, the nerve impulse promotes the synchronous release of many quanta of the transmitter, acetylcholine, the response to which is recorded postsynaptically as the endplate potential (e.p.p.)[2]. Strontium can substitute for Ca^{2+} in the support of transmitter release evoked by nerve impulses[3–5], although it is far less effective (on a molar basis) in the generation of the e.p.p. It has been suggested[5] that Sr^{2+} may be less accessible from the extracellular fluid during the action potential, and might not achieve as high a concentration as Ca^{2+} in the nerve ending so that it would seem less effective. Alternatively, intra-terminal releasing sites may exhibit true selectivity for divalent cations. To help distinguish between these possibilities, I have made use of the long-established observation that when nerve terminals are depolarised by elevating the extracellular potassium ion concentration ($[K^+]_o$), there is a dramatic increase in the frequency of spontaneous miniature endplate potentials (m.e.p.ps)[6–9]. This is thought also to be a form of depolarisation–secretion coupling (that is, mediated by Ca^{2+} entry through voltage-gated channels), though of relatively long duration. I have compared the relative abilities of Ca^{2+} and Sr^{2+} in supporting the augmented m.e.p.p. frequency seen in high K^+ Ringer's solutions and report here that they are qualitatively and quantitatively equivalent, in contrast to their differences in the nerve impulse-evoked e.p.p.

All experiments were carried out on the isolated cutaneous pectoris nerve–muscle preparation of the frog (*Rana pipiens*). Conventional electrophysiological techniques were used for intracellular recording of m.e.p.ps from single endplates. Preparations were washed initially in a standard Ringer's solution of the following composition (mM): NaCl 115, KCl 2, $NaHCO_3$ 6, $CaCl_2$ 1.8; *p*H 6.9–7.2. Before the beginning of an experiment, a preparation was washed for at least 1 h in divalent cation-free Ringer's solution[5]. It was then treated with a Ringer's solution which contained 11 mM KCl and to which were added appropriate amounts of $SrCl_2$ or $CaCl_2$. All solutions contained neostigmine methylsulphate (1 μg ml^{-1}), to increase m.e.p.p. amplitudes. In those experiments where the concentration of divalent cation was varied, osmolarity was maintained constant by substitution of 2.5 mM sucrose for every 1 mM $SrCl_2$ or $CaCl_2$ (ref. 10). The frequencies of m.e.p.ps were determined in a given solution by recording m.e.p.p.s for successive 1-, 2- or 10-s intervals. At least 30 intervals were recorded, from which the mean m.e.p.p. frequency was calculated. Only data from those junctions where the m.e.p.p. frequency in a given solution was relatively stable over time were included in the experimental results.

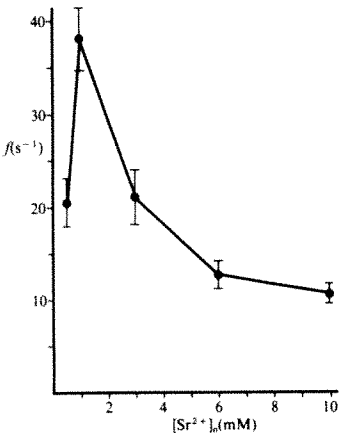


Fig. 1 Effects of $[Sr^{2+}]_o$ on m.e.p.p. frequency (f) at a single endplate in Ringer's solution containing 11 mM K^+. Each point represents the mean ± s.e.m. of 30 1-s counts at a given $[Sr^{2+}]$. The osmolarity was kept constant throughout the experiment by addition of appropriate amounts of sucrose to the Sr^{2+}-Ringer (see text).

Figure 1 shows the results of an experiment in which the effect of external Sr^{2+} concentration ($[Sr]_o$) on m.e.p.p. frequency in 11 mM K^+ Ringer's solution was studied. In this experiment, the m.e.p.p. frequency increased between 0.5 mM Sr^{2+} and 1.0 mM Sr^{2+} and then declined progressively as the $[Sr^{2+}]_o$ was raised, up to 10 mM. This non-monotonic dose–response curve is quite similar to that found previously with Ca^{2+} in similar conditions[8,9,11]. For example, Matthews and Wickelgren found that the relationship between $[Ca^{2+}]_o$ and m.e.p.p. frequency in high K^+ solutions reached a peak at about 1–3 mM Ca^{2+} and declined at concentrations greater than 3 mM. These authors suggested that the inhibitory effects of high Ca^{2+} are due to its screening of the membrane surface charge, with consequent membrane hyperpolarisation and inactivation of voltage-sensitive Ca^{2+} channels. The qualitatively similar behaviour of Sr^{2+} is consistent with such a screening hypothesis, because Sr^{2+}, as a divalent cation, would also be effective in screening the membrane surface charge and in closing down voltage-gated Ca^{2+} channels[12,13]. The question remains, however, as to whether the response mediated by Sr^{2+} is quantitatively similar to that of

Table 1 Comparison of the effects of Ca^{2+} and Sr^{2+} on m.e.p.p. frequency in 11 mM K^+ Ringer's solution

Experiment no.	$[Me^{2+}]$ (mM)	$f_{Ca}(s^{-1})$	$f_{Sr}(s^{-1})$	f_{Ca}/f_{Sr}
1	0.5	5.7 ± 0.5	5.2 ± 0.7	1.10
2	0.5	3.9 ± 1.0	4.7 ± 0.9	0.83
3	0.5	6.8 ± 1.9	5.4 ± 1.2	1.26
4	2.0	22.3 ± 3.0	20.9 ± 3.1	1.07
5	2.0	13.8 ± 1.9	13.7 ± 2.4	1.01
6	5.0	13.9 ± 3.0	15.3 ± 3.2	0.91
7	5.0	5.6 ± 1.1	5.7 ± 1.3	0.98
8	5.0	5.2 ± 0.9	5.6 ± 1.1	0.93
9	7.0	13.4 ± 2.0	12.9 ± 1.7	1.04
10	10.0	2.7 ± 0.7	2.4 ± 0.5	1.12
11	10.0	7.7 ± 1.2	8.2 ± 1.2	0.94
				Mean = 1.02

The m.e.p.p. frequency at a given $[Ca^{2+}]$ (f_{Ca}) was compared at the same endplate with that in equimolar $[Sr^{2+}]$ (f_{Sr}); f_{Ca}/f_{Sr} represents the ratio of the two values, which in no case differs appreciably from unity. Each f value represents the mean ± s.e.m. of 30 successive counts of 2 s duration in experiments 1, 2, 3, 7, 8, 10 and 11 and of 1 s duration in experiments 4, 5, 6 and 9. For each experiment, $[Me^{2+}] \equiv [Ca^{2+}]$ or $[Sr^{2+}]$. Data are presented from 11 different preparations. Note that although there is considerable variation among preparations, the basic non-monotonic trend with respect to divalent cation concentration is evident. This trend is more clearly shown for Sr^{2+} in Fig. 1 and for Ca^{2+} in ref. 9.

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Ca^{2+}, over the concentration range studied in the above experiment. To answer this question, several experiments were carried out in which the m.e.p.p. frequency in high K^+ Ringer's solution containing Ca^{2+} was compared at the same endplate with that in a solution containing equimolar Sr^{2+}. Figure 2 shows a record of such an experiment in which m.e.p.p. frequencies were recorded continuously for approximately 85 min. When the preparation was bathed in a Ringer's solution containing 0.5 mM Ca^{2+} and 2 mM K^+, the mean m.e.p.p. frequency recorded over about 5 min was $1.2 \pm 0.2\ s^{-1}$ (mean ± s.e.m.). When the $[K^+]_o$ was raised to 11 mM, the m.e.p.p. frequency increased to $24 \pm 3\ s^{-1}$. When the bathing solution was changed to one containing 0.5 mM Sr^{2+} (in 11 mM K^+), the m.e.p.p. frequency remained at the same level ($23 \pm 2\ s^{-1}$). On returning to 0.5 mM Ca^{2+}, 11 mM K^+, the m.e.p.p. frequency was again maintained ($25 \pm 2\ s^{-1}$). Finally, when $[K^+]_o$ was reduced again to 2 mM, the m.e.p.p. frequency returned to the control level ($1.4 \pm 0.2\ s^{-1}$). This experiment suggests that at equimolar concentrations, the two ions are equally capable of supporting the increased m.e.p.p. frequency evoked by high $[K^+]_o$. Table 1 presents a summary of experiments done on 11 other preparations in which the two ions were compared in concentrations of 0.5–10 mM. It can be seen that, although there is some variation among preparations, there is essentially no difference between Ca^{2+}-activated and Sr^{2+}-activated transmitter release.

The depolarisation of the nerve membrane produced by treatment with high K^+ is a sustained event, as it is determined primarily by the Nernst equilibrium potential for K^+ (ref. 14).

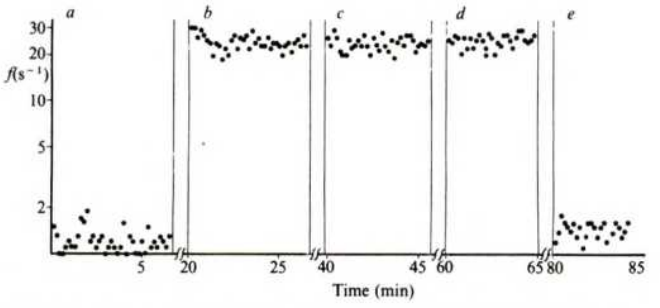


Fig. 2 Augmentation of m.e.p.p. frequency (f) by high $[K^+]_o$ in Ca^{2+} and Sr^{2+} Ringer's solutions. Continuous recording of m.e.p.ps over about 85 min. Each point represents the m.e.p.p. frequency over a 10-s counting interval. Note the logarithmic scale on the ordinate axis. An equilibration time of approximately 15 min for each solution change was allowed before data were collected. *a*, The control frequency (in 0.5 mM Ca^{2+}, 2 mM K^+) was $1.2 \pm 0.2\ s^{-1}$ (mean ± s.e.m.). *b*, In 0.5 mM Ca^{2+}, 11 mM K^+, the frequency increased to $24 \pm 3\ s^{-1}$. *c*, In 0.5 mM Sr^{2+}, 11 mM K^+, the frequency was $23 \pm 2\ s^{-1}$. *d*, On returning to 0.5 mM Ca^{2+}, 11 mM K^+, the frequency remained constant ($25 \pm 2\ s^{-1}$). *e*, On return to the control solution (0.5 mM Ca^{2+}, 2 mM K^+), the m.e.p.p. frequency returned to $1.4 \pm 0.2\ s^{-1}$.

This is distinguished from the depolarisation which occurs during the nerve impulse in that the latter is of short duration (of the order of 1 ms) and therefore allows only a transient increase in Ca^{2+} (or Sr^{2+}) permeability. During this brief event, Sr^{2+} might have a lower mobility in the ionic channel and therefore might not enter the terminal and gain access to the intra-terminal releasing sites to the same extent as Ca^{2+}. In contrast, high K^+ provides a prolonged depolarisation to the nerve ending and as Ca^{2+} channels at the presynaptic membrane probably do not exhibit time-dependent inactivation[15–17], this depolarisation provides a steady-state, long-lasting increase in Ca^{2+} (or Sr^{2+}) permeability. This prolonged event might allow enough time for Sr^{2+} to accumulate at release sites to the same extent as Ca^{2+}. In these conditions, a difference between the two ions might reflect true intracellular ionic selectivity. The experiments described above, however, indicate that when ion entry factors are minimised (by a prolonged increase in permeability), the differences between Ca^{2+} and Sr^{2+} as supporters of transmitter release disappear. This suggests that there is divalent cation non-selectivity at intra-terminal releasing sites. Further support for this view comes from a recent report[18] in which transmitter release was studied in neuromuscular preparations treated with tetrodotoxin and 4-aminopyridine to block the nerve action potential. In these conditions, brief electrotonic depolarisation of the nerve ending seems to trigger a regenerative, 'all-or-none' Ca^{2+} current presynaptically[19] and evoke a giant (>70 mV) e.p.p. postsynaptically. Equimolar substitution of Sr^{2+} for Ca^{2+} gave an e.p.p. of identical amplitude. Thus, even in conditions of brief applied depolarisation, Ca^{2+} and Sr^{2+} seem to be equivalent as supporters of transmitter release, when, as in this case, it is presumed that a maximal ion flux was generated and entry into the nerve terminal ceased to be a limiting factor.

In conclusion, the results of these experiments support the notion that the relatively low efficiency of Sr^{2+} in substituting for Ca^{2+} in nerve impulse-evoked transmitter release does not reflect true intracellular ionic selectivity. Instead, it is probably related to a phenomenon of reduced access of Sr^{2+} to the nerve terminal cytoplasm, because in conditions in which such access ceases to be a limiting factor, the difference in efficiency is abolished.

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Agonist-induced potentiation of acetylcholine sensitivity in denervated skeletal muscle

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The entire surface membrane of denervated skeletal muscle is sensitive to the neuromuscular transmitter, acetylcholine (ACh), whereas in innervated muscle only the junctional area is sensitive. It has been proposed that this difference is due to a 'trophic' effect exerted by ACh in innervated muscle to keep the extrajunctional regions of the surface membrane insensitive to its depolarising action[1]. Several studies[2–4] have demonstrated an agonist-induced potentiation of ACh sensitivity, followed by desensitisation, at the endplate region of normal muscles. The potentiation has been attributed to a cooperative action of ACh on the receptors[3]. Desensitisation of the extrajunctional regions of denervated muscles by ACh has also been described[5]. We now provide evidence that the transmitter itself potentiates the ACh contracture and depolarisation responses of the denervated muscles of the rat *in vitro* and that it produces this effect by increasing the number of available ACh receptors on the surface membrane.

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We used the slow soleus and the fast extensor digitorum longus (EDL) muscles of rats of 100–150 g body weight which had been denervated 4–6 days previously. Muscle contracture and membrane depolarisation responses to diffuse application of ACh were used to test the chemosensitivity of the muscles[6,7]. The contracture reponses to ACh (10^{-6} kg l^{-1}) increased with successive applications of the drug, reaching a maximum after 60–100 min. Figure 1 shows the results from a typical experiment. The maximum increases observed were three to nine times greater than the initial responses to the same dose of ACh ($n = 9$). The magnitude of the twitch tension elicited by supramaximal electrical stimulation remained unchanged throughout the experiment. When carbachol was used to elicit contractures the responses again increased with successive applications of the drug. Carbachol is a cholinergic agonist which is not inactivated by cholinesterases; thus, the increases in the response are not due to inactivation of acetylcholinesterase.

The increases in the contracture reponses with time of incubation were due to a potentiating action of ACh, as when muscles were incubated in Krebs–Henseleit solution for 100 min before ACh application, they exhibited contracture reponses immediately after the preincubation which were comparable in magnitude to the initial responses of muscles which had not been preincubated. Furthermore, the responses of the preincubated muscles increased with successive ACh applications and the time course of the increase was similar to that seen with muscles which had not been preincubated (Fig. 1). As all muscles were stimulated electrically throughout the experiments, including the preincubation period, the ACh potentiation of the contracture responses was not a consequence of muscle contraction.

To determine whether the potentiation of the contracture response was due to an action on the membrane, we measured the depolarisation of the extrajunctional membrane in response to repeated application of ACh (10^{-7} kg l^{-1}) in 4- and 5-d denervated soleus muscles. The depolarisation responses, like the contractures, increased with successive ACh applications, reaching a maximum after 60–100 min (Fig. 2). No significant changes in the resting membrane potential were recorded during the experiments. Values of 65.3 ± 0.8 mV (30 fibres) were recorded before the first application of ACh and 64.3 ± 1.2 mV (30 fibres) after 120–150 min incubation.

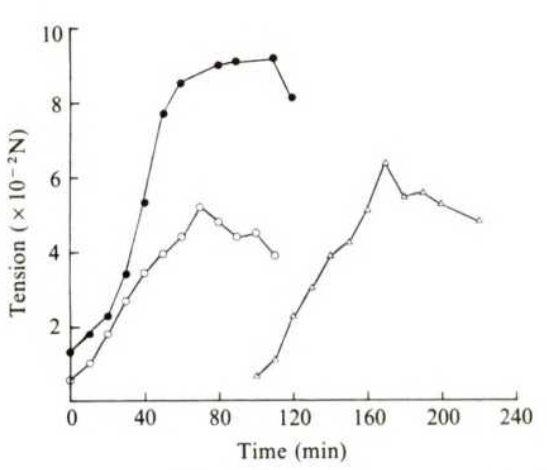


Fig. 1 Potentiation of the ACh contracture responses in denervated muscles. The soleus and EDL muscles of rats denervated 4–6 d previously by unilateral section of the sciatic nerve[6], were dissected out under ether anaesthesia and suspended in an organ bath containing Krebs–Henseleit solution. All muscles were stimulated electrically at 0.1 Hz and supramaximal intensity throughout the experiment. The initial resting tension was adjusted so that the electrical stimulation produced the maximum contracture. The contractures in response to 10^{-6} kg l^{-1} ACh were recorded as described previously[6]. The drug was removed each time by washing the muscle with 10 bath volumes of Krebs–Henseleit solution. ●, EDL; ○, soleus; △, soleus. In this case ACh application was started at 100 min but electrical stimulation was started at zero time.

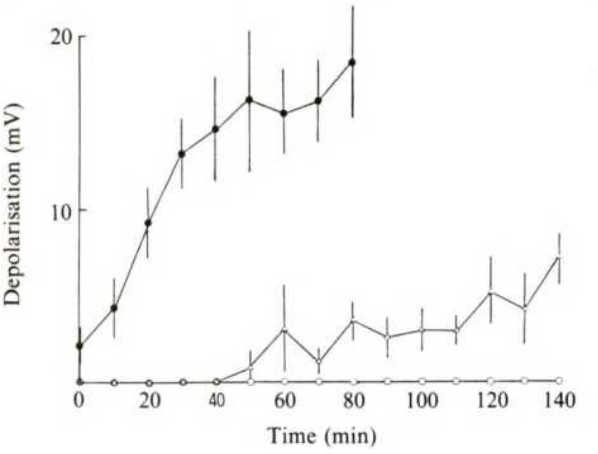


Fig. 2 Effect of ACh on the membrane depolarisation responses of denervated soleus muscles. The resting membrane potential and membrane depolarisation responses to ACh were measured as described previously[7]. Muscles were pinned in an organ bath in Krebs–Henseleit solution and fibres impaled with conventional 3 M KCl-filled micropipettes[12] at extrajunctional regions 2–4 mm from the distal tendons. ACh (10^{-7} kg l^{-1}) was applied diffusely at 10-min intervals, and the drug was removed by washing the muscles with 10 bath applications of Krebs–Henseleit solution. ●, Unblocked muscles ($n = 8$); △, muscles blocked with BTX and then treated with ACh (10^{-5} kg l^{-1}) at 10-min intervals ($n = 5$); ○, control muscles pretreated with ACh (10^{-7} kg l^{-1}) until no further increases in response were seen (10 applications) and then blocked with BTX and treated again with ACh (10^{-5} kg l^{-1}) at 10-min intervals ($n = 5$). Blockade of receptors was effected by incubation of muscles with 0.01 g l^{-1} BTX for 1 h followed by continuous washing with Krebs–Henseleit solution for a further 1 h to remove excess toxin . Values are given as the means with the s.e.m.

To investigate whether the increases in the membrane depolarisation response were due to an increase in the number of receptors available to react with ACh, the effect of the transmitter on muscles blocked with α-bungarotoxin (BTX) was studied. This toxin binds almost irreversibly with ACh receptors[8]. We first established that all available receptors could be blocked, by treating denervated control muscles with ACh until the maximum depolarisation response had been observed, and then incubating the same muscles with the toxin. Such muscles showed no response to ACh subsequently applied at 10-min intervals over 120 min. On the other hand, muscles blocked with BTX in the same conditions but without prior treatment with ACh subsequently exhibited a depolarisation in response to ACh. No responses were seen with the first few applications of the drug but with subsequent applications there was a progressively increasing response (Fig. 2). The new responses were dependent on ACh treatment, as BTX-blocked muscles which were washed for 4 h before ACh applications were insensitive to the first two to four applications of the drug, although small responses were seen with subsequent applications ($n = 4$). The new response could be blocked with BTX in the same conditions as before.

Other workers[9] have demonstrated an increasing contracture response in denervated rat diaphragm blocked with BTX and proposed that BTX alters the membrane architecture, thereby initiating the incorporation of new ACh receptors into the membrane; however, these workers found no effect of ACh on this process. In the present study, the increases in the response in soleus and EDL muscles could be demonstrated regardless of whether or not the muscles had been blocked, and the potentiation was dependent on ACh treatment.

Muscles which have been desensitised by ACh have been reported to bind less BTX than muscles which have not been

treated with ACh[10]. Although it is unlikely that agonist-desensitised receptors are present initially in denervated muscles in view of the absence of ACh and the spontaneous reversibility of this type of desensitisation, it is possible that some receptors are initially held in an inactive conformation, unable to bind BTX or ACh, and can be activated by some action of ACh on the muscle membrane. On the other hand, pools of receptors, possibly of a precursor nature and inaccessible to BTX binding, have been demonstrated in other muscles[11]. Thus, it is possible that the potentiating effect of ACh on muscle chemosensitivity is due to an increase in the number of receptors available to react with ACh or BTX by exposure of previously masked receptors or an incorporation of preformed receptors or receptor precursors into the muscle surface membrane.

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Intracellular *p*H and the sodium requirement at fertilisation

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Several lines of evidence suggest that ionic messengers are primary agents in the metabolic derepression which occurs at fertilisation. The derepression at fertilisation or parthenogenetic activation of the sea urchin egg occurs in two main phases. The first phase, which triggers the early events of fertilisation, is mediated by transitory increase of intracellular free calcium[1–3]. The second, which triggers the late events of fertilisation, is mediated by a rise in the intracellular *p*H (refs 4–6). The transition from the early events of fertilisation of sea urchin eggs to the late events requires a minimal concentration of sodium in the external medium[7]. External Na^+ is required for the acid efflux which follows fertilisation[8]. Na^+ requirement and the acid efflux have been correlated in a hypothesis which proposes that internal protons are exchanged for external Na^+ (refs 8, 9). By using *p*H-sensitive microelectrodes, we have examined the relationship between external Na^+ and internal *p*H more closely. We demonstrate here that the increase of the intracellular *p*H following egg activation does require external Na^+. However, the relative insensitivity of the alkalisation of the egg cytoplasm to large reductions of external Na^+ is evidence against the Na–H exchange hypothesis.

Intracellular *p*H was measured using Thomas-type, *p*H-sensitive microelectrodes with recessed-tips[10]. The calibration and use of these electrodes for sea urchin eggs have been described[11]. As fertilisation by sperm is difficult in the complete absence of sodium[12], we activated the eggs with the calcium ionophere, A23187 (ref. 1). Figure 1 illustrates the change of the intracellular *p*H of unfertilised eggs of *Lytechinus pictus* exposed to A23187 in natural seawater. The alkalisation induced by ionophore activation is similar to that of fertilisation[6]. When eggs were activated by A23187 in Na^+-free artificial seawater (ASW, choline substitution), the elevation of the fertilisation membrane was observed; however, the rise of the internal *p*H did not occur (Fig. 1). When external Na^+ was added back (arrow, Fig. 1), the ctyoplasm underwent alkalisation. Thus, the elevation of cytoplasmic *p*H associated with activation of the egg required external Na^+.

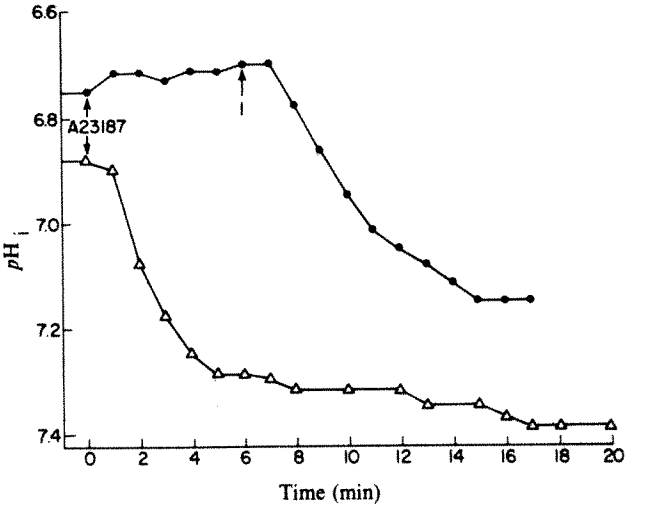


Fig. 1 The external Na^+ requirement for alkalisation after egg activation. In all experiments, the *Lytechinus pictus* egg was impaled with two microelectrodes, The egg membrane potential was measured by a conventional microelectrode filled with 3 M KCl. The *p*H-sensitive microelectrode recorded the membrane potential and a voltage proportional to *p*H. Both electrode measurements were continuous and recorded on separate channels of a Gould-Brush Mark 220 chart recorder. To obtain the intracellular *p*H (pH_i), the membrane potential was subtracted from the *p*H microelectrode potential at intermittent points. Care was taken to assure that any fluctuations of the internal *p*H was plotted. The noise of the recordings was less than 200 μV. The *p*H-sensitive electrode was calibrated in 100-mM phosphate buffers (*p*H 6.5 and 7.5) before and after each experiment. The full response times of these electrodes were 30 s or less. The *p*H of natural seawater (SW) was adjusted with 1 M HCl or NaOH. Artificial seawater (ASW) had the following composition in mM: NaCl, 480; KCl, 10; $CaCl_2$, 5.5; $MgSO_4$, 29; $MgCl_2$, 27; $NaHCO_3$, 5. The *p*H was adjusted with 1 M HCl or NaOH. The temperature was 16–18 °C in all experiments. The activation of eggs by A23187 was in SW, *p*H 8 (△) and in Na-free ASW, *p*H 8 (●). Na-free ASW was made with choline chloride substitution for NaCl, reduction of KCl to 5 mM and $KHCO_3$ substitution for $NaHCO_3$. *p*H was adjusted with 1 M HCl or KOH. At the arrow, ASW was added to the bath to raise the external Na^+ to 80 mM.

It has been reported that amiloride (an inhibitor of passive Na^+-entry into many Na^+-transporting epithelia[13,14]) at 10^{-4} M could block 80% of the acid efflux associated with fertilisation of *Strongylocentrotus purpuratus* eggs when the external Na^+ was reduced to 50 mM (ref. 8). We tested the effects of low external Na^+ and the presence of amiloride on the changes of the intracellular *p*H after fertilisation of *L. pictus* eggs. The addition of 0.5 mM amiloride to 50 mM Na^+-ASW (choline substitution) did not have any apparent effect on cell division of fertilised eggs. However, inhibitory effects of amiloride in 25 mM Na^+-ASW were observed. Amiloride at 10^{-4} M in 25 mM Na^+-ASW did not inhibit the rise of the intracellular *p*H after fertilisation (Fig. 2). These eggs were seen to divide at a rate similar to control eggs in 25 mM Na^+-ASW without amiloride. At $2.5–5 \times 10^{-4}$ M, amiloride inhibited the rate of increase in internal *p*H. These eggs divided 30–60 min after division of control eggs. The delay of cleavage by amiloride between 2.5 and 5×10^{-4} M could be eliminated by washes of natural seawater 20 min after fertilisation. At 10^{-3} M, amiloride in 25 mM Na^+-ASW (glucose substitution, see Fig. 2 legend) blocked the rise of internal *p*H,

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although the elevation of the fertilisation membrane was observed. The inhibition by amiloride at 1 mM was irreversible, as the internal *p*H remained acidic after the removal of amiloride by repeated washes of natural seawater (arrow, Fig. 2).

The reduction of external Na^+ to 25 mM (choline substitution) did not inhibit the alkalisation after fertilisation (Fig. 3). An intracellular *p*H increase in external Na^+ of 5 mM, which is near the minimal external Na^+ required for activation of metabolism after fertilisation[7], has been observed. The *p*H of this unfertilised egg was 6.83 and the membrane potential −18 mV. As the *p*H of the medium was 8, there is a net outward driving force on protons of 50 mV. After fertilisation, the *p*H of the egg was 7.14 and the membrane potential was −73 mV, giving an inward driving force on protons of 23 mV. This reversal of the driving force on protons with egg activation was similar to observations made in natural seawater[6]. As protons are not in electrochemical equilibrium across the egg plasma membrane, the presence of an active mechanism regulating cytoplasmic *p*H is implied. It has been reported that transfer of fertilised eggs to Na^+-free ASW 10 min after fertilisation did not inhibit development[7]. This suggested that external Na^+ was not required for the maintenance of the alkaline *p*H of fertilised eggs. As seen in Fig. 3, the removal of external Na^+ did not affect the internal *p*H. Furthermore, we exposed the eggs to a brief pulse of acidic (*p*H 6.5) Na^+-free ASW. The cytoplasmic *p*H fell rapidly and stabilised at a new value of 6.67. During the acidic pulse, the membrane depolarised to −66 mV, due to a loss of newly developed potassium conductance[15]. The inward driving force on protons had increased to 76 mV. After the bath was returned to Na^+-free ASW, *p*H 8, the cytoplasmic *p*H and membrane potential recovered. The subsequent elevation of the external Na^+ to 80 mM had little effect on either the membrane potential or intracellular *p*H. Because, in the absence of external Na^+, exposure of eggs to acidic medium caused an increase of the inward driving force on protons and the cytoplasmic *p*H recovered following the return to an alkaline medium, the active mechanism regulating the internal *p*H of fertilised eggs was not dependent on external Na^+.

These data demonstrate that external Na^+ is necessary for the rise of the internal *p*H after egg activation. However, once the rise of the internal *p*H has been completed, external Na^+ was not required for maintaining the new alkaline *p*H. As in other tissues, protons are not in electrochemical equilibrium across the cell membrane of the sea urchin egg, implying the existence of an active mechanism regulating the cytoplasmic *p*H. However, the measured net outward driving force on protons of unfertilised eggs may be a consequence of impalement. Because of the high input impedance of unfertilised sea urchin egg membrane[15,16] and the large tip diameters of the *p*H microelectrode, the low resting potential is probably due to a leakage artefact. Careful recordings of the membrane potentials of unfertilised *L. pictus* eggs with a single KCl microelectrode gave membrane potential values near −75 mV (R.A.S., S.A. McClure and S.S.S., in preparation), in close agreement with the values reported for other species of sea urchin eggs[16,17]. If the true values of the internal *p*H and membrane potential of the unfertilised egg are near 6.8 and −75 mV respectively, proton distribution across the plasma membrane would be near passive equilibrium. Alternatively, our reported values of cytoplasmic *p*H of unfertilised eggs may be a consequence impalement and may not reflect the true values. This possibility cannot be totally refuted; however, several lines of evidence suggest that the measured values are near the true values despite the lowered membrane potential. Preliminary experiments indicate that the intracellular *p*H of sea urchin eggs is insensitive to alterations of the membrane potential using voltage clamp techniques and estimation of the internal *p*H of unfertilised eggs by the distribution of labelled 5,5'-dimethyl-2,4-oxazolidinedione (DMO) gave values near 6.8 (ref. 18). This difficulty of leakage artefact is not encountered with the fertilised egg due to its lower input impedance[15,16].

The elevation of the cytoplasmic *p*H associated with activation of the egg required minimal amounts of external Na^+, however, the precise mechanism by which a small amount of sodium permits this alkalisation remains unclear. It has been proposed that the external Na^+ is necessary for a Na–H exchange[8]. Our data on the insensitivity of the alkalisation of the egg cytoplasm to reductions of external Na^+ until the critical threshold concentration and the high concentrations of amiloride necessary for inhibition weakens the Na–H exchange hypothesis. An Na–H exchange has been suggested for the regulation of intracellular *p*H of the mouse soleus muscle fibres. The recovery of the internal *p*H of the mouse soleus muscle fibres following acidification was significantly slowed by an 11% reduction of external Na^+ and blocked by amiloride at 10^{-4} M

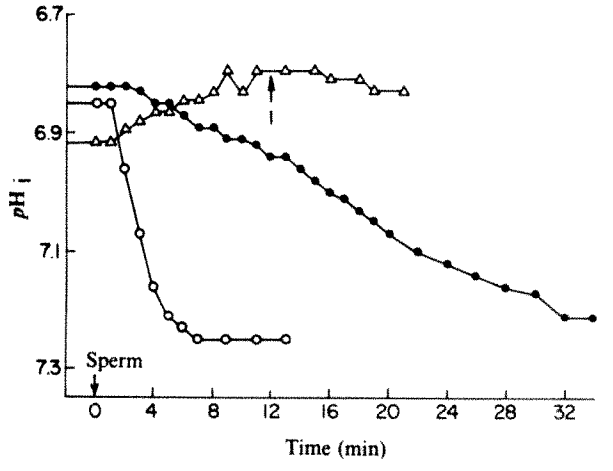


Fig. 2 The effect of amiloride on alkalisation after egg fertilisation. Amiloride at 10^{-4} M (○) had no significant inhibition, at 2.5×10^{-4} M (●) inhibited the rate of alkalisation and at 10^{-3} M (△) blocked alkalisation. Amiloride at 1 and 2.5×10^{-4} M was tested in 25 mM Na^+-ASW, *p*H 8, where choline was substituted for sodium. Amiloride at 1 mM was tested in 25 mM Na^+-ASW, *p*H 8, where glucose (739 mM) was substituted for sodium. No differences in the intracellular *p*H and membrane potentials during fertilisation of controls were observed between glucose and choline replacement for external sodium. Amiloride was obtained from Merck, Sharp & Dohme Research Laboratory and at 1 mM required extensive stirring and slight heating (35 °C), as well as the reduction of the ionic content of ASW. The inhibition by amiloride at 1 mM was not reversed after washing with SW, *p*H 8 (arrow).

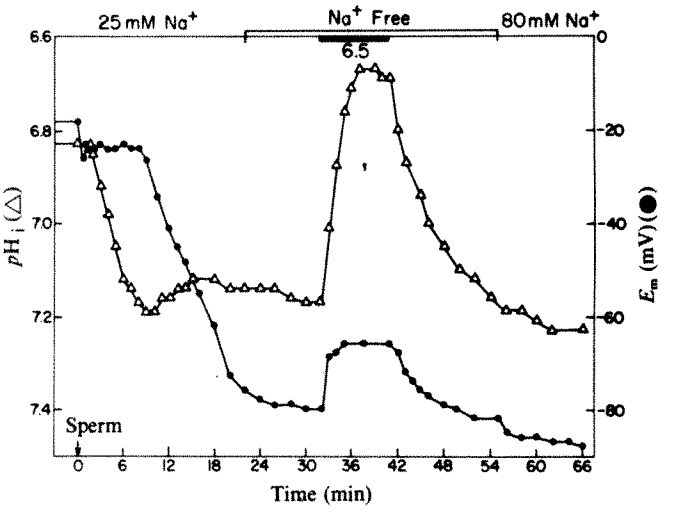


Fig. 3 The effect of external Na^+ and *p*H on the membrane potential (E_m) (●) and the intracellular *p*H (pH_i) (△) after fertilisation. The egg was fertilised in 25 mM Na^+-ASW, *p*H 8 (choline substitution). At 22 min after fertilisation, the external Na^+ was removed. Subsequently, the egg was pulsed for 9 min with Na^+-free ASW, *p*H 6.5. At 55 min, ASW was added to the bath to raise the external Na^+ to 80 mM.

(ref. 19). We have found that the alkalisation after egg activation was not inhibited by a 99% reduction of external Na^+ and inhibition by amiloride only occurred at concentrations in excess of 10^{-4} M with the external Na^+ reduced to 25 mM. It has been reported that amiloride at 2.5×10^{-4} M in 115 mM Na^+-ASW did not inhibit acid production after fertilisation of *Psammechinus miliaris* eggs. However, the rate of acid production was slowed in the presence of the drug[20]. Our data on the relationship between amiloride and external Na^+ concentrations on the alkalisation of the cytoplasm suggested that sodium and amiloride are competitive for binding sites, similar to amphibian tissues[21]. However, the inhibitory concentrations of amiloride necessary is 1,000-fold greater for eggs than epithelia. The effect of such high concentrations of amiloride on eggs may be nonspecific; that is, the effect may be on some process other than ion exchange. As less than 5 mM external Na^+ is required for the elevation of cytoplasmic *p*H associated with egg activation and external Na^+ is not required for maintaining the alkaline *p*H of fertilised eggs, we propose a possible allosteric role for sodium. The binding of sodium to sites on the plasma membrane of the fertilised egg is required for activation of the mechanism directly involved with the alkalisation of the cytoplasm.

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Membrane response to 1-methyladenine requires the presence of the nucleus

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Meiosis is re-initiated in starfish oocytes by the action of the hormone 1-methyladenine (1-MA)[1]. It is thought that the primary trigger is localised at the plasma membrane[2,3], and early changes reported are the activation of a Na^+ pump[4] and variation in membrane potential and conductance[5,6]. We report here that external application of 1-MA results in an irreversible switching of the starfish oocyte membrane from one stable electrical state to another. This induced change requires the presence of the germinal vesicle.

The resting potential of *Astropecten aurantiacus* oocytes ranges from −70 to −90 mV. Increasing $[K^+]_{out}$ by a factor of 10 results in a decrease in the resting potential of 44 mV, approximately as predicted by the Nernst equation for a K^+ selective membrane. At rest, specific membrane resistance may range from 2 to 8 kΩ cm^2 (egg diameter 300 μm). On addition of 10^{-6}M 1-MA to the bath, we observed a depolarisation of about 0.5 mV min^{-1} and a gradual increase in input resistance of 0.5 MΩ min^{-1}. At 15 to 30 min later the membrane switched to a new stable electrical state of −10 to −20 mV; 50–170 kΩ cm^2. In this state, increasing $[K^+]_{out}$ by a factor of 10 did not produce any significant change in the resting potential. Concurrent with the switch, or shortly after, the germinal vesicle was observed to break down. It has been shown elsewhere that sea urchin oocytes have resting potentials in the range −50 to −70 mV, whereas this value in mature eggs is −8 to −16 mV (refs 7, 8). The disappearance of K^+ selectivity after maturation has been reported for amphibians[9,10] and starfish[11].

Offsetting the resting potential of oocytes in the depolarising direction also causes the switch, but the germinal vesicle remains intact. In these cases the switch is reversible (by offsetting in the hyperpolarising direction, or spontaneously). In contrast 1-MA matured oocytes cannot be induced to switch back to the original high potential level. This means the two stable resting states pre-exist in the starfish oocyte, as previously hypothesised[11], and that following maturation only one stable state remains. The current–voltage relationships for the starfish oocyte before and after 1-MA treatment are shown in Fig. 1, together with the switch in potential. The voltage excursion in the absence of 1-MA was limited to prevent the switch occurring. The existence of two stable resting states in the untreated oocyte implies an N-shaped *I–V* curve, with two regions of positive slope conductance connected by a third region of negative slope conductance[11]. Such a curve may only be measured in its entirety in voltage clamp conditions.

We suggest that 1-MA interacts with externally situated plasma membrane structures. This would (1) directly cause the progressive closure of K^+ channels, or (2) cause a leak across the membrane which subsequently depolarises the membrane so closing K^+ channels. In fact, the shape of the curve of the *I–V* relationship for the untreated oocyte (Fig. 1) implies that K^+ channels may be closing due to depolarisation. When the conductance approaches zero the membrane becomes unstable and switches to the low potential state. This resting potential level may be regulated by a different population of ionic channels, which are probably nonspecific. Although the switch can occur either by current injection or by interaction with the hormone, the germinal vesicle only breaks down in the latter

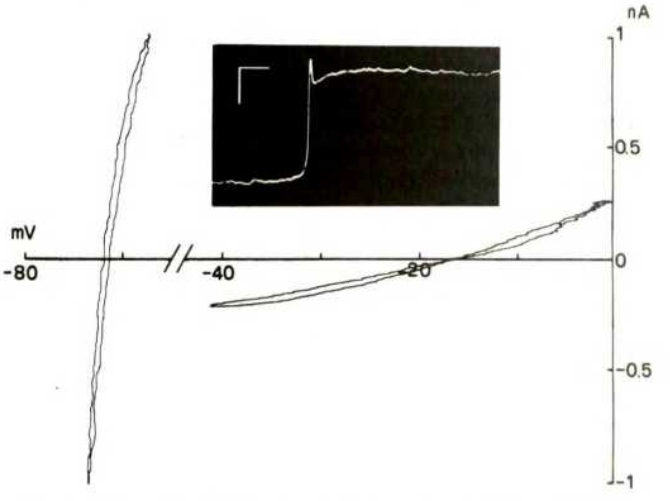


Fig. 1 Recordings from oocytes, complete with jelly and follicle cells, in natural seawater at 20 °C. The inset shows a 1-MA induced switch in an oocyte with an initial resting potential of −80 mV. Horizontal bar represents 5 s, vertical bar 10 mV. The *I–V* curves were obtained by injecting current (*I*), with a sine wave generator (<0.02 Hz), through one intracellular electrode and recording voltage (*V*) with a second intracellular electrode. The curve on the left is from an oocyte before 1-MA treatment, that on the right from the same oocyte after the switch.

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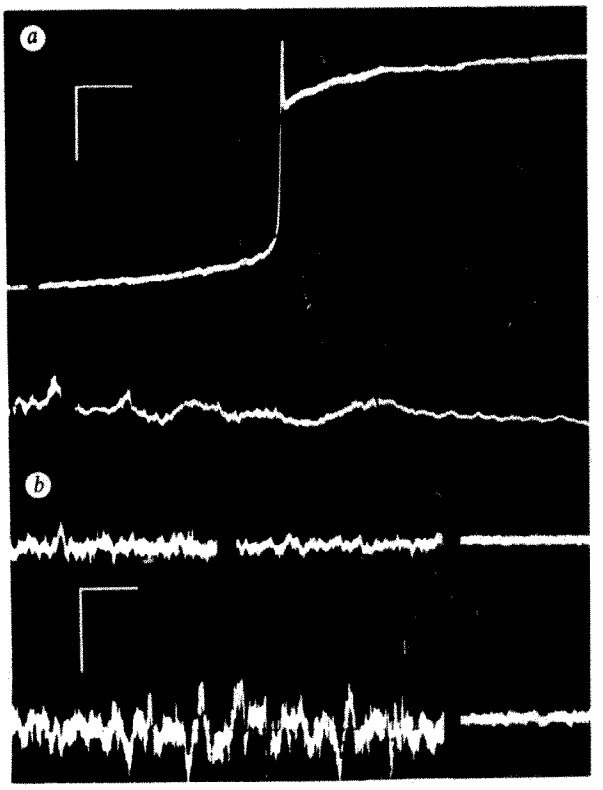


Fig. 2 Anucleated and nucleated fragments were obtained by centrifugation of oocytes in a sucrose gradient at 12,000 r.p.m. or by hand dissection. In each experiment a nucleated and an anucleated fragment of similar size (100–150 μm) were impaled. 1-MA was added to the bath and the responses of both fragments were monitored simultaneously. *a*, The upper trace is from a nucleated fragment which had an original resting potential of −85 mV, and the lower trace is from an anucleated fragment with a resting potential of −95 mV. The horizontal bar represents 5 s, the vertical bar 10 mV. *b*, Voltage noise, a.c. coupled from 1 Hz, in a nucleated fragment (upper) and an anucleated fragment (lower). Upper traces show, from left to right, the spontaneous voltage noise before the switch, after the switch and the microelectrode noise after pulling out the oocyte. Lower traces show, on the left the voltage noise from an anucleated fragment and on the right microelectrode noise after the experiment. The horizontal bar represents 1 s, the vertical bar 0.5 mV.

case. This raises the possibility of the involvement of a secondary factor in the maturation of oocytes.

Starfish oocytes can be divided into nucleate and anucleate halves either manually or by centrifugation. As it is difficult to impale these fragments with a second microelectrode we were unable to study their *I–V* relationships. However, we were able to monitor their responses to 1-MA. Nucleated fragments behaved as intact oocytes. They had stable resting potentials in the range −70 to −90 mV, and 15 to 30 min after 1-MA addition, the switch occurred and the germinal vesicle broke down (seven fragments, upper trace in Fig. 2*a*). The switch also occurred in these fragments by offsetting the resting potential with the recording electrode. The upper traces in Fig. 2*b* show the voltage noise in a nucleated fragment before and after the 1-MA induced switch. Although in this report we have not analysed voltage noise, this characteristic change following the switch seems to be similar in intact oocytes and nucleated fragments. This difference in voltage noise together with the change in specific membrane resistance (observed in intact oocytes) implies a changed population of functional ionic channels and is consistent with our hypothesis.

Anucleated fragments had resting potentials in the range −20 to −100 mV (seven fragments). In addition these fragments, particularly those with high resting potentials, exhibited large slow fluctuations (lower trace, Fig. 2*a*). Addition of 10^{-6}M 1-MA did not induce the switch in anucleated halves, even after exposure for 60 min, and in fact offsetting the resting potential with the recording electrode resulted in a linear relationship between injected current and recorded voltage. Anucleated halves with resting potentials in the range −70 to −90 mV had significantly larger peak-to-peak voltage noise than nucleated fragments (Fig. 2*b*). This could be explained by a difference in input resistance; however, nucleated and anucleated fragments selected for recording were approximately the same size (100–150 μm) and of course the two plasma membranes have a common origin.

At present we have no explanation for the large range in resting potential of the anucleated fragments; however, those with lower resting potentials cannot be described as K^+ selective membranes, whilst those with higher potentials were insensitive to changes in $[K^+]_{out}$.

It has been suggested[2,3] that the primary trigger for 1-MA meiosis re-initiation is localised at the plasma membrane. We have shown that although the initial response is at the plasma membrane, the nucleus must be present for this response to occur. It is possible that a secondary factor controlled by, or contained in, the nucleus might be involved in this hormone induced membrane response. However, the recordings from anucleated fragments suggest that removal of the nucleus, in itself, alters the electrical properties of the membrane precluding the 1-MA membrane response.

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Gonadotropin-releasing hormone analogue binds to luteal cells and inhibits progesterone production

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Although gonadotropin-releasing hormone (GnRH) is believed to mediate the hypothalamic control of pituitary gonadotropin secretion, continuous or repeated administration of GnRH or its agonist analogues has been shown to cause paradoxical antifertility effects in several species, including primates[1–8]. GnRH-induced interruption of reproductive cycles and pregnancy is associated with diminished progesterone production, implying defective function of the corpus luteum[1,3–6]. These luteolytic effects have been attributed[4,5] to the well recognised desensitising actions of elevated luteinising hormone (LH) levels on ovarian LH receptors and steroidogenesis[9,10], subsequent to GnRH-induced gonadotropin release from the anterior pituitary. However, treatment with high doses of exogenous LH did not cause suppression of serum progesterone levels during early pregnancy in rats[8], whereas a highly active GnRH analogue was effective in this regard. These observations suggested that GnRH and its agonist analogues, given in high or sustained doses, can exert a direct action on the ovary that is independent

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of the pituitary. This hypothesis was further supported by the ability of GnRH and its agonists to inhibit human chorionic gonadotropin (HCG)-induced ovarian and uterine weight gain in hypophysectomised rats[11] and to delay the onset of puberty in intact female rats[12]. Also, GnRH and its agonist analogues have recently been shown to inhibit steroidogenesis induced by follicle-stimulating hormone (FSH) in cultured granulosa cells, confirming the direct action of such peptides on the ovarian follicle[13]. The marked inhibitory effects of GnRH and its agonists on corpus luteum function suggest that these compounds could exert direct actions by binding to specific receptors on luteal cells. The present experiments, which examine the effects of GnRH agonists on luteal steroidogenesis, demonstrate the existence of such actions and their mediation by specific high-affinity receptor sites present in luteal cell membranes.

Binding studies with the radioactive GnRH analogue ^{125}I-[D-Ser(tBu)^6]des-Gly^{10}-GnRH *N*-ethylamide (GnRH-A) demonstrated specific uptake of the tracer peptide in both dispersed luteal cells and membrane-rich particles from the luteinised rat ovary. The specific binding of GnRH-A to isolated luteal cells was rapid and saturable, and Scatchard analysis (Fig. 1) indicated that the labelled analogue was bound by a single class of high-affinity receptors ($K_a = 4.4 \times 10^9\ M^{-1}$) presumably located in the plasma membrane. A particulate membrane-rich fraction from ovarian homogenates also bound the labelled analogue, with a comparably high affinity of $5.8 \times 10^9\ M^{-1}$. Binding of the labelled analogue was completely inhibited by 10^{-7} M native GnRH. The binding–inhibition curve obtained with unlabelled GnRH was parallel to that derived with the unlabelled analogue, although for any given displacement a

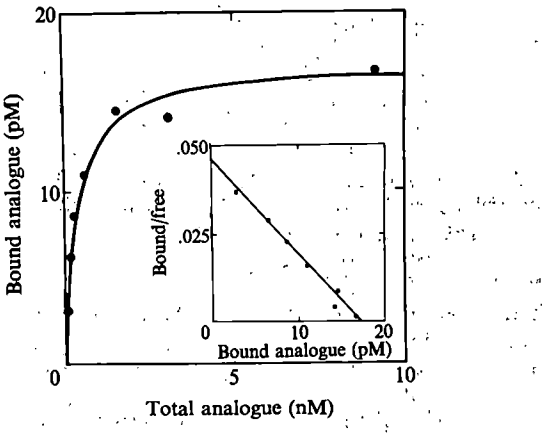


Fig. 1 Binding of ^{125}I-labelled GnRH-A to collagenase-dispersed luteal cells prepared from ovaries of 25-d-old female rats treated with 50 IU PMSG and 25 IU HCG to induce ovarian luteinisation[9]. Animals were killed 8 days after PMSG treatment and isolated cells prepared from the minced ovaries by incubation with 2 mg ml^{-1} of collagenase (Worthington, Type 1 140 U mg^{-1}) in medium 199 containing 0.2% bovine serum albumin (BSA). After 10 min, the collagenase-treated fragments were dispersed by repeated aspiration and filtered through nylon mesh. The resulting cell suspension was preincubated in medium 199/0.2% BSA for 90 min at 36 °C, then centrifuged at 1,500*g* for 10 min. The cell pellet was resuspended in assay buffer (medium 199/0.1% BSA/25 mM HEPES/0.5 mM bacitracin), and triplicate aliquots of 2×10^6 cells were incubated with 3×10^{-11} M ^{125}I-[D-Ser(tBu)^6]des-Gly^{10}-GnRH *N*-ethylamide (1,000 μCi per μg)[15] and increasing concentrations of unlabelled analogue. Incubations were for 80 min at 22 °C in a total volume of 300 μl, and were terminated by dilution with 4 ml of ice-cold phosphate-buffered saline (*p*H 7.4). The cells were immediately separated by filtration through glass fibre filters (Whatman GF/C) and bound radioactivity was determined by γ spectrometry with a counting efficiency of 60% for ^{125}I. Nonspecific binding, assessed in the presense of 3×10^{-7} M unlabelled GnRH-A, was subtracted from all datum points. The total binding when 50,000 c.p.m. of tracer GnRH-A was added was 4–6%, of which 1/5th represented nonspecific binding. The graph depicts a saturation curve and a Scatchard plot (inset) of the data from a representative experiment. Each point is the mean of triplicate determinations which agreed within 10%. The binding affinity, determined from four experiments by computerised Scatchard analysis[14] was $4.4 \pm 0.75 \times 10^9\ M^{-1}$, and the binding capacity was 1.5×10^{-11} M (equivalent to 2,000 sites per cell assuming 75% of cells to be luteal cells).

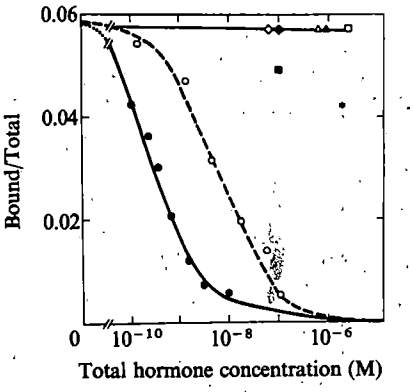


Fig. 2 Specificity of GnRH analogue binding to the 10,000*g* particulate fraction prepared from PMSG/HCG-primed ovaries as previously described for pituitary tissue[14]. The ^{125}I-labelled GnRH analogue (3×10^{-11} M) was incubated with ovarian particles equivalent to 180 μg of membrane protein, with increasing concentrations of unlabelled GnRH-A (●) or native GnRH (○). Incubations were carried out in ice for 80 min in 0.5 ml of assay buffer (10 mM Tris/HCl, *p*H 7.6, containing 1 mM dithiothreitol and 0.1% BSA). Termination of the incubations and separation of the bound fractions were as described for Fig. 1. The equilibrium association constants (K_a) for the GnRH analogue and native GnRH were $5.8 \pm 1.0 \times 10^9\ M^{-1}$ ($n = 3$) and $2.5 \times 10^8\ M^{-1}$, respectively. The binding capacity (receptor concentration) for GnRH-A was 51 ± 12 fmol per mg of ovarian membrane protein. Angiotensin I (▲), angiotensin II (△), thyrotropin-releasing hormone (□), somatostatin (*), ovine LH (◆), ovine prolactin (◇) and HCG (■) (CR 119) were diluted in assay buffer and added at the final concentrations shown.

10-fold higher concentration of GnRH was required (Fig. 2). Thus, native GnRH binds to the same sites as the analogue, albeit with significantly lower affinity ($K_a = 2.5 \times 10^8\ M^{-1}$). Particulate fractions prepared from immature rat ovaries 3 days after priming with pregnant mare serum gonadotropin (PMSG) alone, before the extensive luteinisation induced by HCG treatment, also bound the GnRH analogue with high affinity ($K_a = 9.7 \times 10^9\ M^{-1}$).

The specificity of binding of the labelled GnRH analogue by ovarian receptor sites was examined by competition studies with a series of peptides, as shown in Fig. 2. Angiotensin I, angiotensin II, thyrotropin-releasing hormone and gonadotropic hormones did not inhibit binding of the analogue to ovarian receptors. Only one peptide tested, somatostatin, was slightly inhibitory at high concentrations (10^{-6} M). The tissue specificity of GnRH binding was indicated by the absence of analogue uptake in similar fractions prepared from liver, kidney, adrenal cortex, hypothalamus and cerebral cortex.

To determine the biological function of the ovarian GnRH receptors, the action of the GnRH analogue on progesterone production was examined in isolated luteal cells. In the presence of 5×10^{-7} M GnRH-A, basal progesterone production was reduced by 25% and the stimulating effect of low concentrations of HCG ($< 10^{-13}$ M) was completely inhibited (Fig. 3). In the presence of the GnRH analogue, the maximum progesterone production in response to HCG was similar to that observed in control cells, although a much higher concentration of HCG was required to evoke the maximum steroid response. The ED_{50} for HCG stimulation of progesterone production was increased 25-fold, from 2.5×10^{-13} to 6.0×10^{-12} M, in cells incubated with 5×10^{-7} M GnRH-A. Inhibitory effects of the GnRH analogue, as well as natural-sequence GnRH, were observed at concentrations as low as 10^{-9} M. The inhibitory effect of GnRH peptides on gonadotropin-induced steroidogenesis was not due to interference with hormone binding, as high concentrations of the analogue (up to 10^{-5} M) had no effect on specific uptake of ^{125}I-HCG and ^{125}I-labelled human FSH by ovarian homogenates.

These studies have demonstrated the presence of specific receptor sites for GnRH and an agonist analogue in both isolated luteal cells and particulate membrane-rich fractions prepared from luteinised ovaries. The affinities of the ovarian

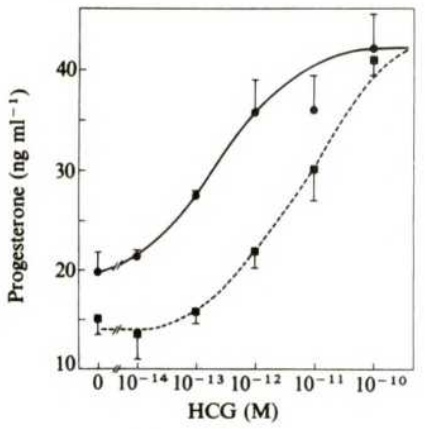


Fig. 3 Effect of [D-Ser(tBu)^6]des-Gly^{10}-GnRH *N*-ethylamide on HCG-induced progesterone production by isolated luteal cells. The cells were prepared as described in Fig. 1 legend, and 2-ml aliquots of cell suspension (2×10^6 cells ml^{-1}) were added to plastic vials containing appropriate dilutions of the hormones. After incubation for 3 h at 36 °C, the suspensions were transferred to glass tubes, centrifuged at 3,000*g* for 10 min, and the supernatant analysed for progesterone by radioimmunoassay[16]. Each point represents the mean ±s.e.m. of values from triplicate incubations. ●, −GnRH-A; ■, +GnRH-A (5×10^{-7} M).

receptors for both GnRH and the highly active analogue were similar to those of the pituitary GnRH receptors for these peptides[14]. However, the GnRH receptor concentration in the ovary was only 20% of that in the pituitary (50 and 235 fmol per mg protein, respectively). Inhibition of progesterone production in isolated luteal cells was observed with low concentrations of both GnRH and its active analogue, consistent with the high binding affinity of the luteal cell receptors. These results suggest that ovarian GnRH receptors mediate the extra-pituitary action of exogenous GnRH and its agonist analogues on progesterone production by the corpus luteum. In addition to their presence in luteinised ovaries, the demonstration of GnRH receptors in ovaries of PMSG-treated rats suggests that such sites mediate the recently reported inhibitory effect of GnRH on FSH-stimulated oestrogen production by granulosa cells[13].

The present observations in ovarian luteal cells, together with those of Hsueh and Erickson[13] in granulosa cells, provide an explanation for the paradoxical effects of GnRH and related compounds on reproductive processes in the female. It is clear that the actions of GnRH agonists are mediated through specific receptors that are separate from the gonadotropin receptors in the ovary, and that such sites are present in both granulosa and luteal cells. The inhibitory effects of GnRH and its analogues cannot be ascribed to interference with gonadotropin binding, thus implying that GnRH and its agonists act at a stage after gonadotropic hormone–receptor interaction. In the present study, the major effect of the analogue was to reduce target-cell sensitivity to gonadotropin, causing a shift in the dose–response curve, with no change in the maximum progesterone production evoked by high concentrations of chorionic gonadotropin. These results indicate that the GnRH analogue acts as a competitive inhibitor of gonadotropin-stimulated progesterone production, at least in short-term studies.

Anti-fertility effects of GnRH and its analogues have been previously observed *in vivo* after pharmacological doses of these peptides. Our findings have demonstrated that receptor-mediated inhibitory actions of GnRH and an active analogue are detectable *in vitro* at low concentrations of the peptides. Because GnRH is undetectable in the systemic circulation, it is unlikely that GnRH of hypothalamic origin could reach the ovary in sufficient concentration to exert a direct action on the corpus luteum. It is conceivable that GnRH or a related peptide is produced in the uterus and transported to the ovary, or is formed locally in the ovary where it acts as a mediator of normal luteolysis. This mechanism, and the possibility that GnRH binds to a heterologous receptor in the ovary in a manner analogous to the morphine/enkephalin interaction, raise intriguing questions about the biological significance of the extra-pituitary actions of GnRH and its analogues.

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Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito

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In the mosquito *Aedes aegypti*, a blood meal triggers egg development. Early workers used the simple technique of decapitation, or neck ligation, to show that a factor from the head was required for normal egg development[1–4]. Lea subsequently demonstrated that the source of this factor was the medial neurosecretory cells of the brain[5]. The hormone from these cells, which he termed the egg development neurosecretory hormone (EDNH), is stored in the corpus cardiacum (CC) and released shortly after a blood meal[6]. Previous work had demonstrated that the ovary begins to produce ecdysone after a blood meal[8]. Considerable evidence suggests that the ecdysone, after being converted to 20-hydroxyecdysone, stimulates the synthesis and secretion of vitellogenin by the fat body[8–12]. The vitellogenin is taken up by the growing oocytes and becomes part of the yolk. Analysis of the amount of 20-hydroxyecdysone present in females after a blood meal showed that significant quantities were first seen 4–6 h after a blood meal[8]. Vitellogenin synthesis begins at about the same time[13]. The early decapitation experiments of Gillett demonstrated that the head was necessary for 4–8 h after a blood meal for egg development to proceed normally[3]. A similar critical time was found for the control of vitellogenin synthesis by the brain hormone[7]. The coincidence of this timing suggested to us that the ovary was the target of the brain hormone. The experiments reported here were designed to test the hypothesis that the brain hormone released after a blood meal acted on the ovary, causing the synthesis of ecdysone. Our results confirm this.

In earlier work we had successfully stimulated vitellogenin synthesis by incubating ecdysone-secreting ovaries with fat body preparations[7]. Our success with this *in vitro* approach prompted us to try to activate ovarian secretion of ecdysone by incubating ovaries with brains. Ecdysone in the medium after incubation was measured using an ecdysone radioimmunoassay[14]. We found that incubating whole brains (not including the CC, as it is removed with the aorta) with ovaries stimulates ecdysone secretion (Table 1). This effect is almost entirely due to the

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Table 1 Effect of parts of the central nervous system on ecdysone secretion by ovaries

	pg ecdysone per 10 pairs of ovaries ± s.e.m.
Aedes aegypti (mosquito)	
Adult female	
Whole brain	510±68
Midbrain	339±39
Optic lobes	0
Suboesophageal ganglia	16±10
Thoracic ganglia	18±0.9
Abdominal nerve cord	46±6.9
Adult male	
Whole brain	496±58
4th instar larva	
Whole brain	206±17
Periplaneta americana (cockroach)	
Larval CC	744±82
Adult CC	505±52
Papilio polyxenes (butterfly)	
Larval brain and CC	163±28
Adult brain and CC	149±17
Musca domestica (fly)	
Adult brain	132±18

Parts of the nervous system from five animals were frozen and thawed and incubated with ovaries from 10 female mosquitoes for 18 h at 25 °C in sterile *Aedes* saline[18] containing 0.15 mg ml^{-1} penicillin and 0.25 mg ml^{-1} streptomycin. This saline is isosmotic with haemolymph[19]. The adults used were 3–5 d old and were maintained on 3% sucrose at 27 °C. The medium and one rinse of the ovaries were combined and assayed for ecdysone using a radioimmunoassay (RIA)[14]. As we have previously shown that ovaries secrete only α-ecdysone when incubated *in vitro*[8], the results are expressed as pg ecdysone per 10 pairs of ovaries. The antiserum used was approximately equally sensitive to α-ecdysone and 20-hydroxyecdysone. Ovaries without any added nervous system gave a background of 40 pg of ecdysone that was subtracted from the above values in this experiment only. Each value represents the mean ± s.e.m. of three replicate studies. No correction is made in these data for the relative size of the brains used.

midbrain; other parts of the nervous system have little or no effect. Brains from adult males and from larvae also stimulate ovaries, although larval brains have half the activity of adult brains. Surprisingly, the CC and/or brains of several related insects activate ecdysone synthesis by mosquito ovaries (Table 1). The CC of larval cockroaches show considerably greater activity than those of adults. Cockroach CC is also much more active than brains and CC from a butterfly or the brains of a fly. The control of ovarian ecdysone secretion by a brain hormone in *Locusta* has been recently described[15] but in this case male or larval brain and CC were not stimulatory.

Saline extracts of whole mosquito heads also stimulate ecdysone production by the ovary. Ecdysone synthesis is linear for 10 h after exposure to head extracts, with a lag of 0.5 h (Fig. 1). In contrast, there is a delay of 6–8 h after a blood meal before the main peak of ecdysone is seen in the whole animal[8]. As the response *in vitro* is almost immediate (Fig. 1), this suggests that release of brain hormone *in vivo* is delayed. Gillett[3] came to the same conclusion by noting the effects of decapitation on egg development. The incubated ovaries are quite sensitive to the factor in head extracts: the amount of hormone present in 1.5–2 heads is sufficient to stimulate a half-maximal response in 10 pairs of ovaries incubated in 50 μl of extract (Fig. 2). At high concentrations of head extract the stimulation of ecdysone is inhibited. This effect has been noted repeatedly and is apparently due to a detrimental factor in the extract that is diluted out more rapidly than the hormone. Heating the extract has no effect on the inhibition.

Evidence that the ovary produces ecdysone *de novo* in response to the brain hormone was obtained by examining the amount of ecdysone in the ovaries and incubation medium before and after exposure to head extract (Table 2). A small amount of ecdysone is detectable in the ovary before the

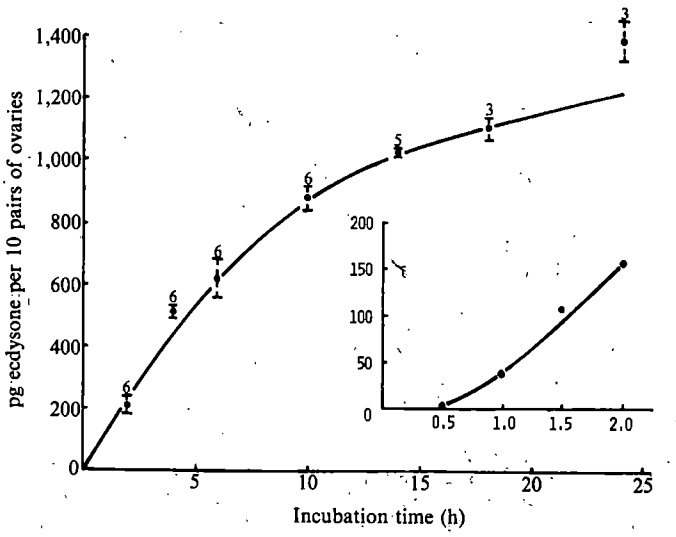


Fig. 1 Time course of ecdysone synthesis in response to head extract. 10 mg of heads were homogenised in 1 ml of sterile *Aedes* saline and treated as described in Table 2 legend except that the extract was not heated. The medium was assayed for ecdysone by RIA[14] after incubation. The data points represent the mean ± s.e.m. The number of replicates is indicated. The inset represents a separate experiment with two replicates, each with 20 pairs of ovaries. Ovary donors were as described in Table 1.

experiment. After 18 h with the head extract, 30 times more ecdysone is found in the incubation medium than in the ovary.

We have demonstrated that the ovary is indeed the target tissue of a brain hormone that stimulates ecdysone synthesis. This hormone is probably identical with the EDNH described by Lea[5,6], as the active factor is found in the region of the brain containing the medial neurosecretory cells (Table 1). A major unsolved problem concerns the nature of the stimuli that cause the release of EDNH after a blood meal. Neither the act of feeding nor stretching of the abdomen during feeding seems to be important[6]. There is some evidence that a humoral agent released after blood feeding is involved; this may be an early product of blood meal digestion[16].

EDNH is analogous in function to PTTH, the prothoracicotropic hormone found in immature insects. Both cause the synthesis of ecdysone by their respective target tissues; the function of the ecdysone in immature insects is to cause moulting. The similarity may be more than analogous, given that

Table 2 Synthesis of ecdysone by ovaries in response to head extract

	pg ecdysone per 10 pairs of ovaries ± s.e.m.
Ovaries before incubation	14
Saline extract before incubation	0
Ovaries after incubation	35
Saline extract after incubation	1,100±25

Heads were obtained from 5–10-d old adult mosquitoes maintained on 3% sucrose by shaking animals frozen at −60 °C to break them apart and separating out the heads using US standard sieves. Heads were retained by a mesh size of 0.425 mm, lyophilised and 5 mg (36 μg per head) homogenised in 1 ml of sterile *Aedes* saline[18]. The extract was then sonified three times for 0.5 min and centrifuged for 5 min at 9,000*g*. The extract was heated at 50 °C for 15 min and centrifuged again. Heating effects some purification but does not significantly reduce the activity of the extract. Samples of supernatant (50 μl) were incubated with 3 groups of 10 pairs of ovaries each. After 18 h the ovaries were removed and combined to provide enough material for the RIA. Also 30 pairs of ovaries that were not incubated were dissected. The ovaries were homogenised and extracted with 50% methanol. The supernatant after centrifugation for 5 min at 9,000*g* was dried down and assayed for ecdysone by RIA[14]. The saline extract before and after incubation was also analysed for ecdysone. Ovary donors were as described in Table 1.

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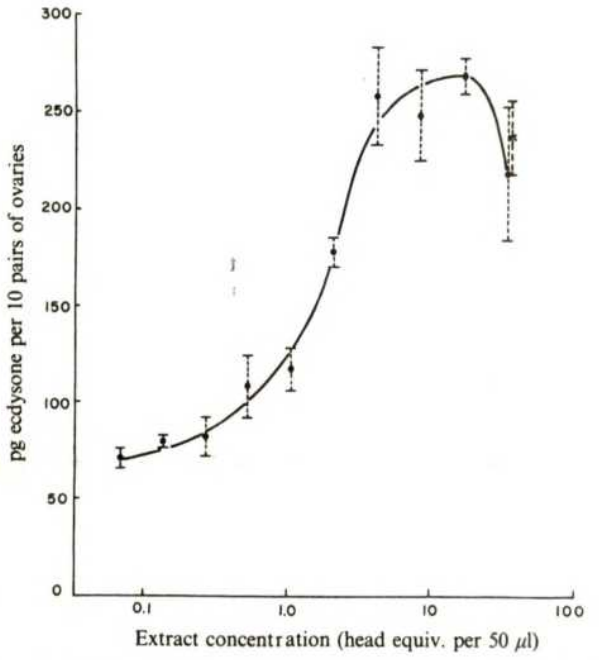


Fig. 2 Ecdysone synthesis by ovaries in response to increasing amounts of head extract. 20 mg of mosquito heads obtained as described in Table 2 legend were homogenised in 0.8 ml of sterile saline[18]. The extract was frozen and thawed and then centrifuged for 30 min at 10,000*g*. The sample was divided, one part was serially diluted and the other heated at 50 °C for 15 min. Ovaries from 10 7-d-old females were incubated with 50-μl samples of the extract for 6 h at 25 °C. Ecdysone was determined in the incubation medium by RIA[14]. The results were converted to head equivalents by the factor, 1 head = 36 μg. Data given are mean ± s.e.m. of five replicates. X Indicates the heat-treated sample.

EDNH activity is present in larval mosquito brains and the brains and CC of several other insects. It is possible that these two hormones are structurally similar and therefore homologous to some degree. Work in our laboratory on the chemistry of EDNH has shown it to be a peptide of molecular weight about 6,500 with some similarities to PTTH from *Bombyx mori*[17].

The neuroendocrine control of ecdysone synthesis by the mosquito ovary resembles in several ways the control of oestrogen synthesis by the vertebrate ovary. In both, a peptide produced by neurosecretory cells or the pituitary stimulates the synthesis of a steroid by the ovary. Perhaps the cyclic production of eggs that occurs in both is best accomplished by this type of control mechanism.

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A circadian oscillator in cultured cells of chicken pineal gland

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The activity of serotonin *N*-acetyltransferase, the key enzyme of melatonin synthesis, shows a marked circadian rhythm in the pineal glands of various animal species[1–10]. The regulation mechanism of the *N*-acetyltransferse rhythm in birds is different from that in mammals. *N*-Acetyltransferase activity in rat pineal gland is controlled by the central nervous system through the sympathetic nerves from the superior cervical ganglion[1–6], while in chicken the endogenous oscillator for *N*-acetyltransferase rhythm is presumably located in the pineal gland. Recently it has been shown that *N*-acetyltransferase activity oscillates in a circadian manner in the organ culture of chicken pineal glands[11–13]. When chicken pineal glands were organ-cultured under continuous illumination, the nocturnal increase of enzyme activity was suppressed. These observations suggested that chicken pineal gland contains a circadian oscillator, a photoreceptor and melatonin-synthesising machinery. A central question arises whether the circadian oscillation of *N*-acetyltransferase activity and its response to environmental lighting are generated within the cell or are emergent properties of interaction between different types of pineal cells. I report here that in the dispersed cell culture of chicken pineal gland, *N*-acetyltransferase activity exhibits a circadian rhythm and responds to enviromental lighting in the same manner as in the organ culture.

Chickens (White Leghorn cockerels) were raised under the lighting schedule with lights on from 7 a.m. to 7 p.m. for 12–14 days after hatching. After decapitation, their pineal glands were dissociated by treatment with collagenase. More than 90% of the cells were viable as judged by Trypan blue exclusion. Shortly after the treatment with collagenase, most of the cells were dispersed and there was little cell–cell attachment. The typical morphological appearances of the cultured cells are shown in Fig. 1. After inoculation in dishes, some of the cells tended to reaggregate into small clusters. Within 24 h fibroblasts had developed processes. Round cells were alive and mostly attached to fibroblasts, although some remained isolated.

When dispersed cells were cultured in a light–dark cycle (LD 12 : 12), both the specific activity (per mg protein) and the total activity (per dish) of *N*-acetyltransferase were 5–10-fold higher during the dark periods than in the light periods on days 2 and 3 of culture (Fig. 2*a*, *b*). As the protein content increased, with doubling time of about 1.5 days, the total enzyme activity showed a more marked difference between day and night-time.

Table 1 Effect of conditioned medium on *N*-acetyltransferase activity in cell cultures

Medium added	*N*-acetyltransferase pmol per mg protein per 10 min
Medium unchanged	313 ± 17
Daytime conditioned medium	344 ± 41
Night conditioned medium	361 ± 22

Conditioned medium was taken from the 3-day cell culture during daytime (3 p.m.) or at night (3 a.m.) in darkness, and was added to other 3-day cell cultures next day at 11 a.m.. The incubations were continued until 5 p.m. and *N*-acetyltransferase activity was measured. The results are means ± s.e.m. of five samples.

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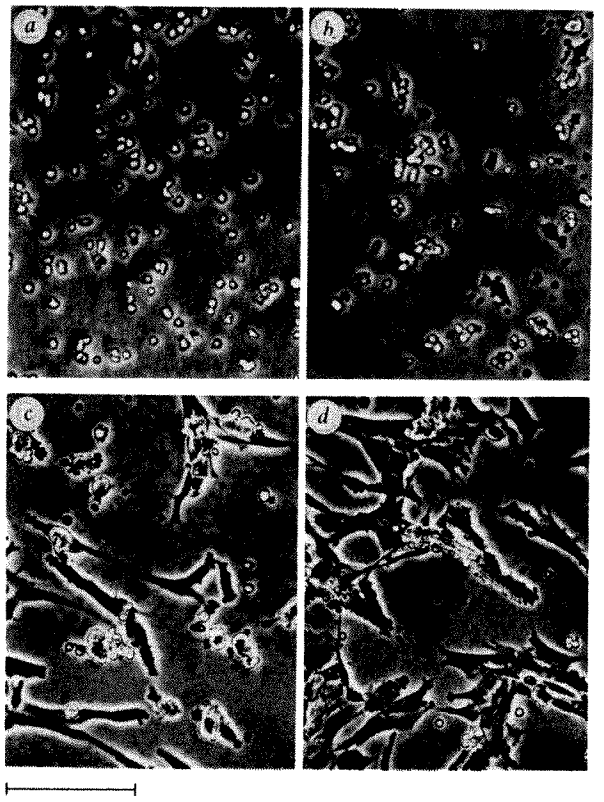


Fig. 1 Phase contrast micrographs of dispersed pineal cells shortly after inoculation (*a*), after 24 h (*b*), 48 h (*c*), and 60 h (*d*) of culture. Scale bar, 100 μm. Eighty to ninety chickens were killed between 2 and 3 p.m. by decapitation. The pineal glands were freed of connective tissues and severed into small pieces by pincettes under a stereo microscope. The pineal tissues were incubated in a 40-ml tube containing 25 ml of incubation mixture at 37 °C with constant shaking. The incubation mixture consisted of 25 mM HEPES–NaOH (*p*H 7.2), 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 10 mM glucose, 0.36 mM $CaCl_2$, streptomycin (100 μg ml^{-1}), penicillin (100 U ml^{-1}), 1% bovine serum albumin, and 0.4% collagenase (type I) from *Clostridium histolyticum* (Sigma). During the incubation of 15–20 min, the pineal pieces were triturated by splashing the suspension against the tube wall through a thin Pasteur pipette. Undispersed fragments of the tissues were sedimented by standing the suspension for 10–15 min. The cell suspension was transferred into another tube and the cells were collected by centrifugation at 1,000*g* for 5 min. The cells were washed with 30 ml of culture medium 3 times followed by centrifugation. Suspension (4 ml) containing $1.4–1.6 \times 10^6$ cells was inoculated in a Falcon plastic culture dish (5 cm diameter). Culture medium was the same as that used for organ cultures[11]. Monolayer cultures were carried out at 40 °C in a humidified atmosphere of 10% CO_2 and 90% air in a transparent incubator box placed in a CO_2 incubator. Inside of the incubator was kept dark. When indicated, a flourescent lamp (30 W) and electric bulb (100 W) provided light through a glass door of the incubator. Intensity of the illumination was 800–1,100 lux at dish level. The culture medium was changed every 3 days.

After 3 days of culture, fibroblasts covered most of the surface of dishes, and the specific enzyme activity per mg protein decreased, although the total enzyme activity per dish was maintained for another several days (data not shown).

To examine whether the rhythmic change of *N*-acetyltransferase activity was due to a synchronised cell cycle, cytosine-1-β-D-arabinofuranoside (1×10^{-5} M) was added to the medium on day 3 of culture to inhibit cell division (Fig. 3*b*). The activity of *N*-acetyltransferase still showed a 10-fold increase at night compared to the daytime value. In another experiment, cells were cultured for 14 days (17 days after the start of culture) in the presence of cytosine-1-β-D-arabinofuranoside. *N*-Acetyltransferase activity continued to oscillate rhythmically (on day 17 of culture, daytime values at 4 p.m., 304 ± 15; night values at 4 a.m., 2,733 ± 160 pmol per mg protein per 10 min).

Dispersed cells were cultured under the LD cycle for 2 days and on the third day the lights were turned off at 1 p.m., 6 hours before the onset of usual darkness (Fig. 3*c*). *N*-Acetyltransferase activity did not increase during the subjective daytime, but increased at 10 p.m., reaching a maximum at midnight. In the experiment shown in Fig. 3*d*, cells were cultured in continuous darkness from the second day for two successive days. There were rhythmic changes of two cycles with a higher enzyme activity during the subjective night-time compared to that of the subjective daytime. However, the day–night difference was damped out on the third day of culture: the daytime levels being higher and the night-time values being lower than those of the cells cultured in the LD cycle (Fig. 3*a*).

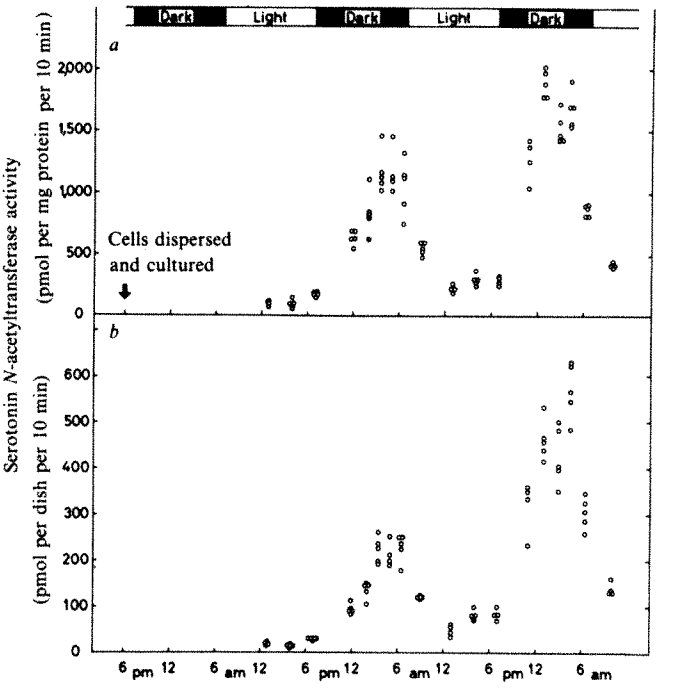


Fig. 2 *N*-Acetyltransferase activity in the dispersed cells cultured in a light-dark cycle. Cultured cells were scraped with a rubber policemen, transferred in a centrifuge tube together with the culture medium, and collected by centrifugation at 1000*g* for 10 min. The pellet was homogenised in 65 μl of 0.05 M potassium phosphate buffer (*p*H 6.5) by use of a small glass homogeniser. The homogenate (50 μl) was used for the assay of *N*-acetyltransferase activity[11] and the rest (15 μl) for the determination of protein by the method of Lowry *et al.*[17]. *a*, Specific enzyme activity (pmol per mg protein per 10 min); *b*, total enzyme activity (pmol per dish per 10 min). Each point represents the enzyme activity of individual dish.

When cells were exposed to continuous illumination in the third night, the increase of enzyme activity was suppressed to half or less (Fig. 3*e*). However, there was threefold elevation of *N*-acetyltransferase activity during the subjective night-time compared to the daytime level. These observations indicate that the rhythmic change of *N*-acetyltransferase activity in the dispersed cells is not a reaction to the environmental lighting, but is generated by an endogenous oscillator in the cells. Cells were cultured for 3 days in the LD cycle inverted to the lighting schedule in which the chickens had been raised. The rhythmic change of *N*-acetyltransferase activity was entrained to the reversed lighting schedule in which the cells were cultured (Fig. 3*f*).

To determine whether one type of pineal cell released a transmitter or a hormone which stimulated the synthesis of

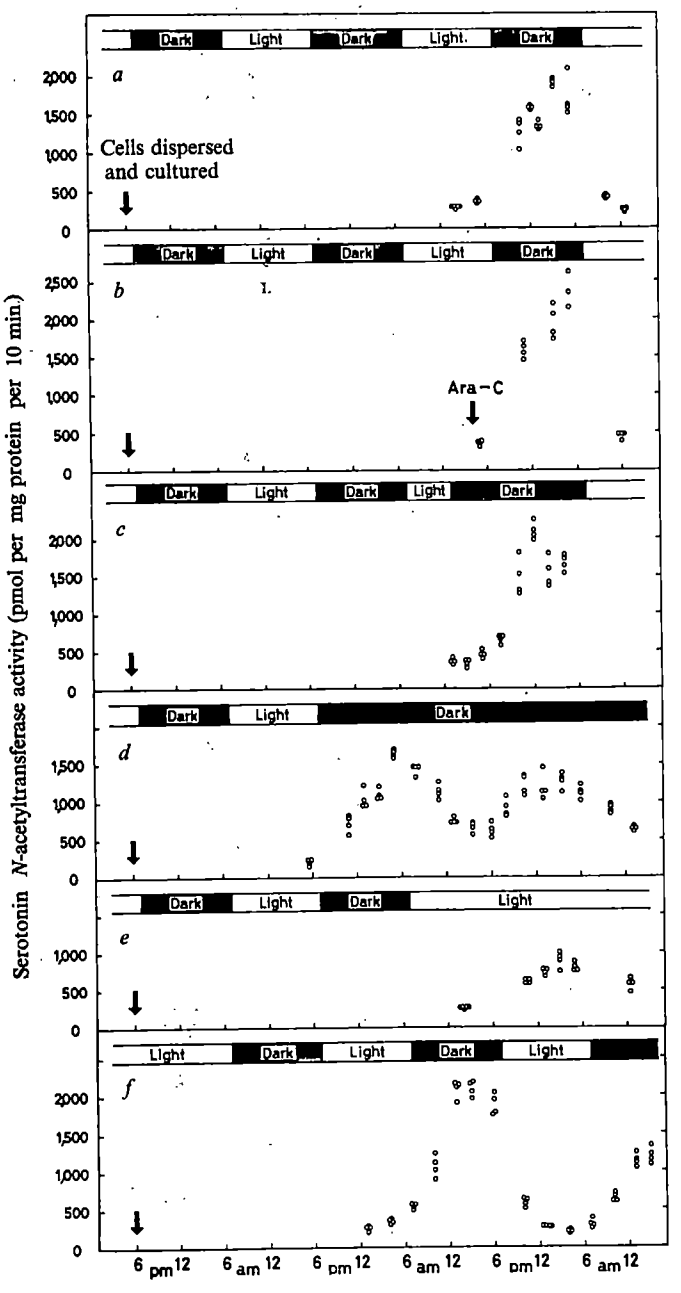


Fig. 3 *N*-Acetyltransferase activity in the pineal cells cultured under various lighting schedules. Each point represents the specific enzyme activity of an individual dish. 'Ara-C' indicates the addition of cytosine-1-β-D-arabinofuranoside (1×10^{-5} M) to the medium.

N-acetyltransferase in another type of pineal cell, conditioned medium was taken from the 3-day cell culture during daytime and at night in darkness, and was added to another cell culture at 11 a.m. when *N*-acetyltransferase activity was low. There was no significant elevation of *N*-acetyltransferase activity in both conditioned mediums (Table 1).

The present observations with the dispersed cell culture are remarkably comparable to those reported using organ culture of chicken pineal gland[11]: (1) In a LD cycle, *N*-acetyltransferase activity continued to oscillate in phase with the lighting schedule for long periods—at least 3 days in the organ culture[11] and 17 days in the dispersed cell culture. However, the amplitude of the rhythm was gradually decreased in the organ culture, presumably due to a necrosis of the gland, while in the dispersed cells the same magnitude of *N*-acetyltransferase activity was maintained during the period examined. (2) When cultured in continuous darkness, the rhythmic changes of *N*-acetyltransferase activity were damped out in both the organ culture and the cell culture. This could be due to desynchronisation of the rhythms in individual cells. (3) Even when lights were turned off during the daytime, *N*-acetyltransferase activity did not increase during the subjective daytime in both organ culture and the cell culture, indicating the endogenous nature of the rhythmicity. (4) Continuous illumination of the glands or the cells suppressed the night-time increase of *N*-acetyltransferase activity, although there were still significant elevations of enzyme activity during the subjective night-time, even under illumination. (5) The rhythmic change of *N*-acetyltransferase activity was entrained to the reversed LD cycle in the dispersed cells, although this was not examined with the organ culture. (6) The night-time level of *N*-acetyltransferase activity in the organ culture was about 3,500 and 1,250 pmol per mg protein per 10 min (700 and 250 pmol per mg pineal per 10 min) in the first and second nights of culture, respectively[11]. The night-time level of enzyme activity in the dispersed cells reported here is 1,500–2,700 pmol per mg protein per 10 min, which is the same as that of organ culture and is close to the *in vivo* rhythm[11]. The daytime activity in the dispersed cells was also comparable to those of intact pineal gland. If the *N*-acetyltransferase rhythm in the dispersed cells is due to the formation of cellular interaction in small clusters, the maximum enzyme activity should be much lower than those of intact glands, because only a portion of the cells form clusters (Fig. 1). To further rule out the possiblity that the reaggregated cells re-establish the specific cell–cell interaction to induce the circadian rhythm of *N*-acetyltransferase activity, dispersed cells were inoculated at different cell populations from 5×10^5 to 4×10^6 cells per dish to give different degrees of reaggregation. Activity of *N*-acetyltransferase showed a similar night-time increase at various cell populations (data not shown).

It has been well demonstrated that, although dispersed embryonic cells reconstruct tissue-like structure in suspension or rotation cultures, the cells rapidly lose the histoformative tendency in monolayer cultures[14–16]. Cells from embryos at a late stage of gestation or from postnatal animals do not show tissue formation in cultures. Thus it is unlikely that the dispersed pineal cells studied here re-established the tissue-like cellular interaction, which in turn generated the *N*-acetyltransferase rhythm. The present observations that the dispersed cells behave in the exactly same manner as the intact pineal glands are very indicative, though not conclusive, that the photoreceptor, the circadian oscillator and the melatonin-synthesising machinery reside in the same cell of chicken pineal gland. This system will offer an ideal model for a long-term study of the circadian oscillator of vertebrates in culture.

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Induction of vitellogenin and growth of implanted oocytes in male cockroaches

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Vitellogenins are yolk protein precursors that are synthesised in the liver of lower vertebrates in response to ovarian hormones[1,2], and in the fat body tissue of insects, under the influence, in most species, of juvenile hormone (JH) from the corpora allata (CA)[3,4]. Vitellogenins are normally restricted to females, although in male amphibians[1] and roosters[2] their synthesis can be induced artificially by the injection of oestrogens. Thus female specificity is maintained by hormonal differences between adult males and females. In insects, on the other hand, because the CA of adults of both sexes are active[4,5], it appeared that male fat body could not normally respond to JH by synthesising vitellogenin. However, precise JH synthetic rates of male CA are only known in *Schistocerca gregaria*[6] and *Diploptera punctata*[7], in which species they are low compared to the rates in the female glands. The absence of vitellogenin in adult males could thus be due to inadequate JH titres. We report here that synthesis of vitellogenin can indeed be induced in males of *Diploptera* by implantation of female CA or application of a JH analogue, ZR512 (Zoecon), and that implanted oocytes take up the vitellogenin.

In preliminary experiments we found that removal of the CA of newly-emerged (day 0) females ($n = 5$) prevented the appearance of vitellogenin on day 6, as determined by rocket immunoelectrophoresis. Also, normal adult males from 6 to 21 days old ($n = 39$) contained no detectable vitellogenin (titres approaching 0.1% that of normal day 6 females, or 10 ng, are detectable by this technique). A pair of CA from day 0 females was then transplanted to day 0 males with or without a single ovary, also taken from day 0 females. Haemolymph of the males was subsequently checked for vitellogenin on days 3–10 by rocket immunoelectrophoresis. We also measured vitellin in the implanted oocytes (after uptake into oocytes, vitellogenin is referred to as vitellin). Vitellogenin was detected in the haemolymph of the CA recipients from day 3 or 4 onward, and reached a peak concentration on day 8 (Fig. 1*a*). In those males that received only CA implants, vitellogenin titres were about twice those of males that received both CA and an ovary. This suggested that the ovary may have removed some of the protein from the haemolymph. Indeed, Fig. 1*b* shows that oocytes contained vitellin, which reached a maximum level by day 9, when approximately 9 μg per ovary was measured. The oocytes themselves grew, with visible yolk granule formation, and had lain down chorion by day 8. However, they were slightly smaller and contained less vitellin than either oocytes of sham-operated females or those implanted into ovariectomised females (Table 1).

When 150 μg of ZR512 was topically applied in 5 μl acetone to day 0 males implanted with an ovary, vitellin levels were much higher by day 8, approaching those of a normal female ovary (Fig. 2*b*). This is consistent with previous findings from this laboratory (unpublished) that ZR512 is a more potent promoter of oocyte growth in allatectomised females than the topically applied naturally occurring JH of *Diploptera* (identified as C_{16}JH ≡ JH III (ref. 8)). On the other hand, haemolymph titres of vitellogenin in ZR512-treated males were comparable to those of males implanted with CA (Fig. 2*a*), suggesting that the limiting step in appearance of vitellin is the synthesis of the yolk protein precursor by the fat body and not its uptake by the oocytes. When acetone alone was applied, no vitellogenin or vitellin was detected and implanted oocytes did not grow.

This is the first conclusive evidence outside Diptera on the induction of vitellogenin in adult male insects. Limited growth of oocytes implanted into males of the cockroach, *Periplaneta americana*, has been reported[9,10] although no vitellogenin

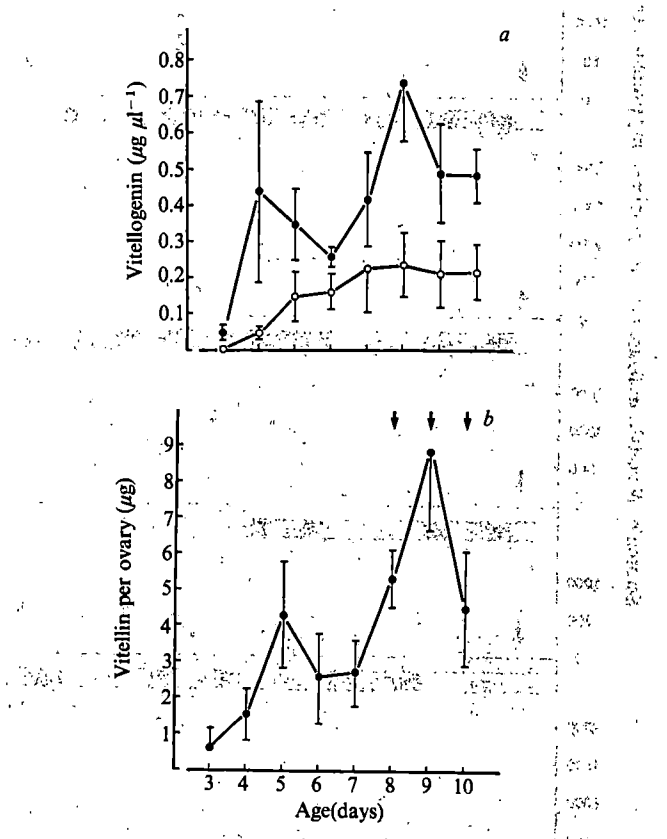


Fig. 1 *a*, Haemolymph vitellogenin concentration in adult males implanted at day 0 with CA of day 0 females, with (○) and without (●) one day 0 ovary (Ov). *b*, Total vitellin in implanted oocytes. The arrows indicate age at which 100% of the oocytes were chorionated. Each point represents the mean of 4–6 determinations (n) except day 10 when $n = 11$. Vertical bars represent one standard error of the mean. Corpora allata were dissected free of adhering fat body tissue and implanted into the neck region of the male recipients. Ovaries were implanted into the abdominal cavity by injection in a small volume of Yeager's Ringer through the intersegmental membrane using a glass needle. Vitellogenin and vitellin titres were determined by rocket immunoelectrophoresis[26]. Antiserum against egg homogenates was raised in rabbits and was rendered specific to vitellin by absorption with male haemolymph. Specificity of the absorbed antiserum was demonstrated by a single immunoprecipitation reaction in agarose against both vitellin of egg extracts and vitellogenin of female haemolymph after separation in polyacryamide gels. Vitellogenin and vitellin were found to be immunologically identical by Ouchterlony double diffusion. Absorbed antiserum was mixed with melted agarose (1% in Tris-buffered 0.025 M Tricine, *p*H 8.6; Bio-Rad) at 50 °C for a final concentration of 0.25–1.00% (v/v), depending on titres of antigen to be tested. Slabs (1.5 mm thick) were then cast, wells cut, and 10-μl samples applied. Electrophoresis was carried out for 12 h at 150 V in a water-cooled chamber (Miles). Immunoprecipitation rockets were quantitated using egg extracts of known protein concentrations as determined by a micro-biuret procedure[27]. Vitellin makes up ~90% of the soluble egg extract (E.C.M., S.S.T. and B.S., in preparation).

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synthesis was detected[10]. Similar results were obtained with *Eublaberus posticus*[11]. In another cockroach, *Nauphoeta cinerea*, both ovaries and female CA were implanted into males but no oocyte growth resulted[12]. After transplantation of oocytes to males of the Lepidoptera, *Bombyx mori*[13,14] and *Pieris brassicae*[15], oocyte development took place, but again, no vitellogenin was detected. Conversely, in *Hyalophora cecropia*, vitellin was detected in implanted oocytes[16]. This finding led to the discovery of minute quantities of the yolk protein precursor in normal adult males (0.1% that of females)[16]. Similar quantities were detected in *Oncopeltus fasciatus*[17].

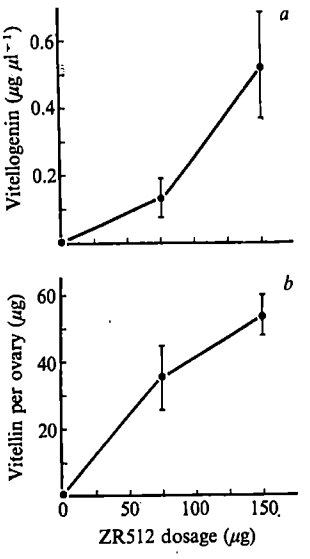


Fig. 2 *a*, Haemolymph vitellogenin concentration in 8-day-old adult males implanted with one ovary at day 0 and topically treated with ZR512 in 5 μl acetone at doses of 75 μg ($n = 8$) and 150 μg ($n = 9$), and with acetone alone ($n = 8$). Procedure as for Fig. 1. *b*, Total vitellin in implanted oocytes on day 8. Procedure as in Fig. 1.

In the Dipterans, *Sarcophaga bullata* and *Drosophila melanogaster*, vitellogenin synthesis in adult males can be induced[18,19]. In *Sarcophaga*, injection of ecdysone reportedly led to the appearance of vitellogenin, whereas JH had no effect. However, implanted oocytes did not mature[18]. In contrast, males of *Drosophilia* were induced to synthesise vitellogenin by implantation of ovaries and oocytes apparently matured completely[20–22]. Synthesis of vitellogenin in *Drosophilia* is reported to require a factor from the ovaries[19] and the ovaries themselves may be a source of vitellogenin[23]. The appearance of vitellogenin in *Diploptera* reported here occurred even when ovaries were not present. Clearly, in Diptera, yolk protein synthesis is controlled differently, both with regard to the role of JH and that of the ovaries.

It is evident that the adult male fat body of *Diploptera* has the capacity to synthesise vitellogenin when stimulated by sufficient titres of JH. It has also been reported that normal adult males of *Rhodnius prolixus* produce yolk protein precursor[24,25], often in amounts similar to females (Engelmann and Mundall, unpublished). These findings, together with the identification of small amounts of vitellogenin in *Hyalophora*[16] and *Oncopeltus*[17] adult males, suggest that the capacity of males to synthesise vitellogenin is not anomalous among insects.

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Table 1 Total vitellin on day 7 in ovaries (Ov) re-implanted into 0 day ovariectomised females (OvX) and those of sham-operated controls*

Treatment	Vitellin (μg per ovary)	*n*
OvX+Ov	84.4±16.2	5
sham-operated	83.2±11.9	5

Values are mean±s.e.m.; *n*, numbers of animals.
* Haemolymph vitellogenin titres were not determined.

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Is multiple sulphatase deficiency due to defective regulation of sulphohydrolase expression?

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Multiple sulphatase deficiency disease is an unusual autosomal recessive disorder characterised biochemically by a deficiency of several sulphohydrolase activities[1,2]. The laboratory diagnosis of this combined neurological connective tissue disorder is made on the basis of decreased activities of the lysosomal enzymes, arylsulphatase A and arylsulphatase B and the microsomal enzyme, arylsulphatase C. The primary defect in this multienzyme deficiency has not been identified. Using immunological techniques to characterise further the residual activities of arylsulphatases A and B in the multiple sulphatase deficiency disease, we have examined the levels of cross-reacting material (CRM) to arylsulphatases A and B in cultured skin fibroblasts from controls and patients with multiple sulphatase deficiency, metachromatic leukodystrophy (deficiency of only arylsulphatase A activity) and Maroteaux–Lamy syndrome (deficiency of only arylsulphatase B activity). We report here results indicating that arylsulphatases A and B in multiple sulphatase deficiency are reduced in their levels of CRM while retaining a normal activity/CRM ratio. Because the two enzymes are apparently structurally unrelated, these data are consistent with the possibility that their combined deficiencies in this disorder may result from a defect in the coordinated expression of sulphohydrolases.

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Skin fibroblasts from patients with multiple sulphatase deficiency ($n = 3$), metachromatic leukodystrophy ($n = 2$) and Maroteaux–Lamy syndrome ($n = 2$), as well as controls ($n = 5$), were grown as previously described. Cultures were also maintained in HEPES-CO_2 as described by Fluharty *et al.*[3], who reported that with growth in this medium, arylsulphatases A and B in multiple sulphatase deficiency returned to normal activity levels: we failed to confirm this observation. Cells were collected by trypsinisation at confluency and lysates containing arylsulphatases A and B were obtained by freeze–thaw and centrifugation. Enzyme assays confirmed the diagnosis of multiple sulphatase deficiency; compared with control, lysates from multiple sulphatase deficiency cell line 1 had <1% arylsulphatase A, ~5% arylsulphatase B; those from multiple sulphatase deficiency cell line 2 had ~7% arylsulphatase A, ~10% arylsulphatase B; and those from multiple sulphatase deficiency cell line 3 had ~5% arylsulphatase A, ~10% arylsulphatase B; arylsulphatase C was 40–60% of control in these lines. In some experiments, arylsulphatase A and B activities were separated by anion exchange chromatography[2]. No cross-reactivity was observed between purified arylsulphatase A and arylsulphatase B to anti-arylsulphatase A and anti-arylsulphatase B, respectively, and therefore intact lysates were also used. Ouchterlony double diffusion demonstrated single lines of identity between normal fibroblast lysates and lysates from multiple sulphatase deficiency cells when tested with either anti-arylsulphatase A or anti-arylsulphatase B. These results validated the use of more quantitative tests to measure the relative levels of CRM to arylsulphatases A and B in the various cell lines.

Figure 1 shows the results of single radial immunodiffusion (SRID) assays[4]. Figure 1*a* plots the anti-arylsulphatase A as ring area against activity. Lysates from multiple sulphatase deficiency cells fell on the same line as controls and Maroteaux–Lamy syndrome cells, whereas the slope for lysates from metachromatic leukodystrophy cells was steeper, indicating considerably less enzymatic activity per CRM than controls, multiple sulphatase deficiency or Maroteaux–Lamy syndrome. Figure 1*b* plots the anti-arylsulphatase A assay ring area against protein in the assayed samples. The multiple sulphatase deficiency lysate fell below the line obtained for control, metachromatic leukodystrophy and Maroteaux–Lamy syndrome lysates. Figure 1*c*, *d* illustrates the data obtained for SRID assays carried out with anti-arylsulphatase B. The data are plotted as described above for anti-arylsulphatase A, with similar results in both plots for two multiple sulphatase deficiency lysates. In this assay, Maroteaux–Lamy syndrome lysates showed considerably lower activity/CRM ratios than controls, multiple sulphatase deficiency and metachromatic leukodystrophy (Fig. 1*c*). Taking all the results of Fig. 1 together, the following conclusions may be made. (1) Arylsulphatase A and arylsulphatase B from multiple sulphatase deficiency cells are similar to controls with respect to enzymatic activity per unit CRM; (2) arylsulphatases A and B are reduced in their CRM levels (in approximate proportion to the decrease in the activity observed in lysate enzyme assays) and, in contrast, (3) the arylsulphatase A in metachromatic leukodystrophy lysates and the arylsulphatase B in Maroteaux–Lamy syndrome lysates are markedly reduced in the CRM-specific enzymatic activity, although CRM to each enzyme is found at normal levels in the cells.

To confirm the observation that the CRM/activity ratio of arylsulphatases A and B in multiple sulphatase deficiency cells was comparable to that observed with normal enzyme, a sensitive competitive precipitin assay was carried out (see Fig. 2 legend for assay method). In Fig. 2, the curves described as 'expected' (×) in each graph represent the anticipated values of residual supernatant activity if the multiple sulphatase deficiency activities have a normal ratio of activity to CRM. Values significantly above expected would indicate the presence of higher amounts of CRM than indicated by the level of enzymatic activity. For both arylsulphatases A and B, values at all antibody concentrations were very close to expected. Identical agreement to expected was also obtained when the level of multiple sulphatase deficiency activity was one-half the normal activity in the assay. These results confirm the findings of the SRID assay in that the enzyme which is present in multiple sulphatase deficiency cells seems to be normal in its ratio of activity to CRM and thus, by implication from this assay, is reduced in the level of arylsulphatases A- and B-specific CRM in the cell.

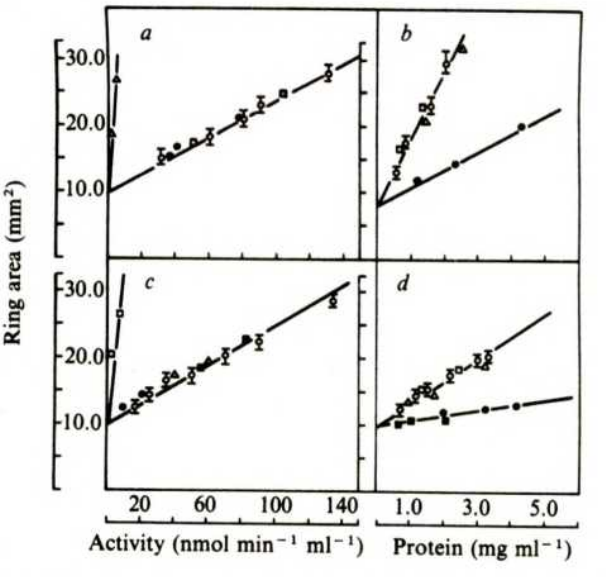


Fig. 1 Single radial immunodiffusion (SRID) assays of fibroblast lysates from controls and patients with multiple sulphatase deficiency, metachromatic leukodystrophy and Maroteaux–Lamy syndrome. Antibodies to purified human liver arylsulphatase A and arylsulphatase B were raised in rabbits by multi-site injection with Freund's complete adjuvant. Arylsulphatase A and B activities were assayed as previously described[9]. Arylsulphatase B was purified as previously reported[10], and arylsulphatase A was purified as follows. Liver was obtained at autopsy, homogenised in 10 mM Tris-HCl, *p*H 7.4 (2:1 v/w), frozen and thawed three times, sonicated briefly, and filtered through gauze; the homogenate was mixed at 4 °C with DE-cellulose equilibrated with the above buffer (1 g DE-cellulose per 2 g wet weight tissue). The adsorbed fraction, free of arylsulphatase B, was eluted batchwise with 1.0 M NaCl, the *p*H was adjusted to 6.0 and the fraction was mixed at 4 °C and 2 g concanavalin A bound by CNBr (ref. 11) to 200 ml Sepharose. The adsorbant was then poured into a column, washed with 20 mM Tris-acetate, *p*H 6.0 and 0.5 M NaCl, and enzyme was eluted with 1.0 M α-CH_3-glucoside, *p*H 7.4, at 22 °C. Activity fractions were concentrated by negative pressure ultrafiltration, dialysed against 20 mM Tris-acetate, *p*H 6.0, and applied to a column of Cibacron Blue–GioGel 0.5 M (100 cm × 2 cm)[12]. Peak fractions were pooled and fractionated on a DE-cellulose column, equilibrated in 10 mM Tris-HCl, *p*H 7.6, and eluted by a 0–500 mM NaCl linear gradient. Final purification was accomplished by fractionation on a column of BioGel P-150 (90 cm × 1.0 cm) at *p*H 7.4 (50 mM Tris-HCl). Purity of the enzyme was demonstrated by polyacrylamide electrophoresis[13] and a symmetrical peak of constant specific activity on gel filtration. Antibodies to arylsulphatase A and arylsulphatase B and their respective IgG fractions prepared by precipitation of the antiserum with 40% saturated ammonium sulphate, appeared monospecific by immunoelectrophoresis against the original liver material. These assays were carried out in Petri plates of 1.5% agarose (12 ml) containing 225 μg ml^{-1} anti-arylsulphatase A IgG or 125 μg ml^{-1} anti-arylsulphatase B IgG and 0.02% NaN_2; 7-μl samples were applied to each well and allowed to diffuse for 48 h at 37 °C. Plates were washed in phosphate-buffered saline for 72 h and, to increase the sensitivity of the assay, a second antibody, goat-anti-rabbit IgG, was applied to each well and allowed to diffuse in a similar manner. Antibody–antigen precipitin rings were measured with a Bausch and Lomb calibrated eyepiece. *a*, *b*, SRID using anti-arylsulphatase A (anti-ASA); *c*, *d*, SRID using anti-arylsulphatase B (anti-ASB). Scale for protein represents actual values for SRID with anti-arylsulphatase A and standardisation to 1.0 mg ml^{-1} for anti-arylsulphatase B (actual values = ×3). ○, Controls (±range, $n = 4$); ■, multiple sulphatase deficiency, cell line 1; ●, multiple sulphatase deficiency, cell line 2; △, metachromatic leukodystrophy; □, Maroteaux–Lamy syndrome. (The results from two mutant lines are pooled for metachromatic leukodystrophy and Maroteaux–Lamy syndrome.)

To examine the possibility that the intracellular activities of arylsulphatases A and B in multiple sulphatase deficiency are reduced because of excessive secretion into the media, control and multiple sulphatase deficiency fibroblasts (cell lines 2 and 3) were examined for levels of arylsulphatase A and B activities in serum-free media in conditions previously described[5]. No differences in arylsulphatase A or B activities were observed between control and multiple sulphatase deficiency cells (~10 nmol per min per mg cell protein). In addition, no differences were observed in the migration of arylsulphatase A and B activities from control and multiple sulphatase deficiency lysates on polyacrylamide gel electrophoresis at *p*H 4.1.

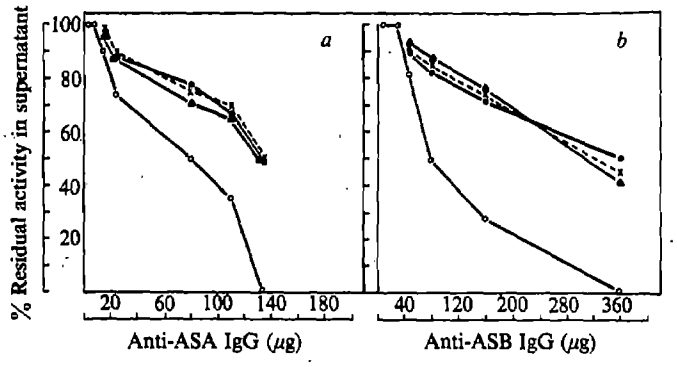


Fig. 2 Competitive precipitin assays of multiple sulphatase deficiency compared with control fibroblast lysates. A precipitin curve for normal enzyme was first established. Increasing amounts of antibody were added to a constant amount of cell lysate (that is, a constant level of activity), incubated first at 37 °C for 120 min then at 4 °C for 16 h. A previously formed complex (100 μg) of human serum albumin (HSA)–anti-HSA was added to each tube as a carrier protein, the mixture centrifuged at 10,000*g* for 20 min and the supernatants assayed for unprecipitated enzymatic activity. Equal levels of enzymatic activity from multiple sulphatase deficiency lysates were then added to tubes containing the previously defined ratio of antibody to normal cell lysate. *a*, Assay of arylsulphatase A using anti-arylsulphatase A; *b*, assay of arylsulphatase B using anti-arylsulphatase B. ○, Control; ●, multiple sulphatase deficiency, line 2; ▲, multiple sulphatase deficiency, line 3; ×, expected values (see text for discussion).

Hypotheses to explain the defect in MSD include (1) a mutation in a subunit or 'factor' common to the sulphohydrolases; (2) the presence of an inhibitor of the affected sulphohydrolases derived from a defect in another metabolic pathway; (3) a defect in a cellular process which coordinately regulates (pre- or post-translationally) the expression of sulphohydrolases. The demonstration that catalytically normal arylsulphatases A and B are present in reduced concentrations in multiple sulphatase deficiency is most consistent with the third hypothesis; these findings are also supported by recent immunological studies by Fluharty *et al.*[3], who observed reduced levels of CRM to arylsulphatase A in multiple sulphatase deficiency. It cannot be determined, however, whether arylsulphatases A and B are decreased in their synthesis or increased in their rate of degradation; the latter may occur, for instance, through a mutation in a putative stabiliser molecule common to the enzymes, a defect which may result in a decay of the sulphatases at both the antigenic and activity levels. These findings in multiple sulphatase deficiency are in contrast to the apparent mutation in the structural genes for arylsulphatase A in metachromatic leukodystrophy and arylsulphatase B in Maroteaux–Lamy syndrome[6]. The recent localisation of arylsulphatase A to chromosome 22 and arylsulphatase B to chromosome 5, as well as the independent expression of each enzyme, provides evidence against a common structural feature between the two enzymes[7]. It is difficult to reconcile our findings with either of the first two explanations. Although mucolipidosis II (I-cell disease) is characterised by the intracellular deficiency of several lysosomal enzymes in cultured skin fibroblasts, the majority of these enzymes may be accounted for by their elevated levels extracellularly[8]. Thus, these studies suggest the existence of a unique monogenic disorder in humans which is the result of a defect in a process which coordinates the expression of several enzymes.

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Inefficient lactate dehydrogenases of deep-sea fishes

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The respiratory rates of deep-sea animals are extremely low. Deep-sea fishes may consume oxygen at rates only 5–10% those characteristic of shallow-water species[1–4]. These low metabolic rates are probably an adaptation to the presumed scarcity of food in deeper water[4]. The biochemical basis of this metabolic adaptation is a low level of enzyme activity in the skeletal muscle (but not the heart or brain), mainly because of the low enzyme concentrations in that tissue[5]. We report here, however, that a second source of reduced enzyme activity contributes to the low metabolic rate. For muscle-type (M_4) lactate dehydrogenases (LDH, EC 1.1.1.27, NAD^+:lactate oxidoreductase), the enzymes of deep-sea fishes have significantly higher activation free energy (ΔG^*) and enthalpy (ΔH^*) characteristics than the homologous enzymes of cold-adapted, shallow-water fishes. Because of these higher energy barriers to catalysis, pyruvate is reduced to lactate at approximately 60% of the rate observed with LDHs of shallow-water fishes. Thus, in terms of rate of function per enzyme molecule, deep-sea fishes would be at a disadvantage in shallow waters because of their relatively poor capacity for muscle glycolysis. Such enzymatic factors may help determine the upper distributions of deep-sea species, much as the relatively large pressure insensitivities of LDHs of these deep-sea fishes[6,7] may enable them to tolerate high and variable pressures. We suggest that the low catalytic efficiencies of high-pressure-adapted LDHs are concomitant with their low sensitivities to pressure.

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Studies of homologous enzymes from animals adapted to different temperatures have shown that ΔH^* and ΔG^* are lowest for reactions of the most cold-adapted species[8–12]. These differences influence reaction rates and are viewed as adaptive, as the lower energy barriers in cold-adapted animals facilitate metabolic rate compensation to temperature. Because the temperatures of the deep sea average only 2–5 °C (ref. 13), one might predict, on the basis of temperature adaptation studies, that deep-sea animals would possess highly efficient enzymes. For M_4-lactate dehydrogenases this expectation is not realised (Fig. 1, Table 1). The Arrhenius activation energies (E_a) of the deep-sea species are homogeneous (analysis of variance (ANOVA), $F_{[3,19]} = 0.99$, $0.5 < P < 0.25$), and the E_a of the shallow-living, cold-adapted species are homogeneous ($F_{[1,19]} = 2.24$, $0.25 < P < 0.10$). However, the E_a values of the deep and shallow species groups differ ($F_{[1,19]} = 9.09$, $P < 0.01$). The M_4-LDHs of deep-sea teleosts belonging to four families are consistently found to have higher activation energy characteristics and lower rates of activity per enzyme molecule (Table 1; see legend for computation procedures) than the M_4-LDHs of shallow-living, cold-adapted species. When compared at 5 °C and 1 atm pressure, M_4-LDHs of deep-sea fishes reduce pyruvate to lactate at only about 60% of the rate of LDHs of cold-adapted, shallow-living fishes (Table 1). Thus deep-sea fishes seem to have a low capacity for anaerobic glycolysis, in comparison with shallow species, in conditions of low hydrostatic pressure and low temperature. However, at deep-sea pressures, the reduced pressure sensitivities of M_4-LDHs of deep-sea fishes may compensate for the low efficiencies of these enzymes[6,7]; at these pressures M_4-LDHs of shallow-living fishes show significant velocity inhibition and perturbation of K_m values[6,7].

The importance of hydrostatic pressure as a selective factor in enzyme evolution is shown particularly clearly by the M_4-LDHs of the two *Sebastolobus* congeners, *S. alascanus* (shallow) and *S. altivelis* (deep). These two species are very similar morphologically and ecologically[6,14], but they experience different pressure regimes at their depths of maximal abundance (Table 1). The difference in habitat pressure (of the order of 50–100 atm, corresponding to differences of 500–1,000 m in depths of distribution) seems to be large enough to involve selection for altered activation parameters, as well as for different pressure sensitivities in K_m values for pyruvate and NADH[6,7]. Despite these significant differences in kinetic properties, M_4-LDHs of the two species could not be distinguished by any of the electrophoretic procedures we reported previously[6]. Evidently, such electrophoretic identity may belie important functional differences between homologous enzymes.

There could be several possible explanations for reduced catalytic efficiencies of enzymes of deep-sea animals. First, if reduced metabolic rates in such a food-poor environment are selectively advantageous, then selection might favour enzymes having relatively low rates of activity[15]. However, we do not support this interpretation, as reduced metabolic activity could be achieved more economically by maintaining lower concentrations of more efficient enzymes. Comparisons of muscle enzyme activities (including LDH) in deep- and shallow-living fishes have shown that enzymatic protein concentrations decrease rapidly as a function of the species' minimal depths of occurrence[5]. The approximately 60% reduction of LDH activity due to inefficiency of this enzyme in deep-sea fishes could well be achieved by enzyme dilution in the cells, as an approximately 25- to 50-fold reduction in LDH activity has already been

Table 1 Activation energy parameters and relative absolute velocities of M_4-lactate dehydrogenase reactions of differently temperature- and pressure-adapted fishes and a mammal

Species* (Depth range/body temperatures)	E_a† (cal mol^{-1})	ΔH^* (cal mol^{-1})	ΔS‡ (cal mol^{-1} K^{-1})	ΔG^*‡ (cal mol^{-1})	Relative velocity‡
Pagothenia borchgrevinki (surface/−2 °C)	11,020 (197)	10,467	−12.7	14,000	1.00
Sebastolobus alascanus (180–440 m/4–12 °C)	11,068 (196)	10,515	−12.6	14,009	0.98
Sebastolobus altivelis (550–1,300 m/4–12 °C)	12,538 (216)	11,985	−8.1	14,249	0.64
Coryphaenoides acrolepis (1,460–1,840 m/2–10 °C)	12,366 (220)	11,813	−8.7	14,222	0.67
Halosauropsis macrochir (~2,300 m/2–5 °C)	12,396 (259)	11,843	−8.6	14,227	0.66
Antimora rostrata (1,300–2,500 m/2–5 °C)	13,110 (223)	12,557	−6.4	14,343	0.54
Thunnus thynnus (bluefin tuna) (surface <300 m/15–30 °C)	11,937 (153)	11,384	−10.0	14,152	0.76
Rabbit (37 °C)	13,100	12,550	−6.4	14,342	0.54

* The depth ranges given for the species are the depths of maximal abundances (see refs 4, 6 and 7 for additional data). For the analysis of variance tests, *P. borchgrevinki* and *S. alascanus* were the shallow-living species, and *S. altivelis, H. macrochir. A. rostrata* and *C. acrolepis* were the deep-living species.

† The Arrhenius activation energy (E_a) values were computed from the slopes, determined by linear regression, of plots such as those shown in Fig. 1. Standard errors for the E_a values are given in parentheses beneath the E_a values. The activation enthalpy (ΔH^*) values are for 5 °C ($\Delta H^* = E_a - RT$).

‡ Estimates of ΔS^* and ΔG^* were computed using the following rationale. For all sets of homologous enzymes that have been studied to determine activation parameters, a highly regular covariation between ΔH^* and ΔS^* has been found[8–12]. For LDHs a plot of ΔH^* against ΔS^* gives a slope of approximately 330 K (refs 9, 11). This slope is called the 'compensation temperature' (ref. 20) to emphasise that simultaneous increases in ΔH^* and ΔS^* have compensating–offsetting effects on $\Delta G (\Delta G^* = \Delta H^* - T\Delta S^*)$. We assume that all of the M_4-LDHs used in this study fit the compensation relationship noted for other vertebrate M_4-LDHs. On this assumption we have estimated ΔS^* values, using experimentally determined differences in ΔH^*, and the experimentally determined ΔS^* from ref. 10 for flounder M_4-LDH (the ΔH^* reported for the latter enzyme is 10,500 cal mol^{-1}). For each 330 cal mol^{-1} increment in ΔH^*, the compensation relationship predicts a 1 entropy unit (cal mol^{-1} K^{-1}) increase in ΔS^*. Thus, using the flounder M_4-LDH reaction as a baseline, we have computed the other ΔS^* values and, using these, the ΔG^* values. The relative velocity values are the reaction rates computed from the differences in ΔG^* among the M_4-LDHs, using the reaction rate of the *Pagothenia* M_4-LDH reaction (the reaction having the lowest ΔH^* and ΔG^* values) as the reference (value = 1.0). The calculated relative rates agree extremely well with experimentally determined rates for the M_4-LDH reactions of *Sebastolobus alascanus* and *S. altivelis*. The specific activities of these two enzymes, in μmol NADH oxidised to NAD$^+$ per mol LDH per min, are 4.66×10^{10} and 2.82×10^{10}, respectively at 5 °C and 1 atm pressure. Protein concentrations were determined by the technique of Sedmak and Grossberg[22], using bovine serum albumin as a standard. Our decision to estimate ΔG^* values using the compensation relationship rather than through direct experimental measurement of velocities and protein concentrations is based on the fact that small amounts of enzymatically inactive protein can greatly distort specific activity values, whereas a high degree of precision is possible in the measurement of ΔH^* values. The regression coefficients for all Arrhenius plots were greater than 0.996. Our value for the ΔH^* of the *Antimora rostrata* M_4-LDH reaction is in excellent agreement with the value reported by Baldwin *et al.*[15]; they obtained values for E_a and ΔH^* of 13,200 and 12,648 cal mol^{-1} (at 5 °C), respectively. Their experimentally determined substrate turnover number for the *A. rostrata* enzyme (2.48×10^{10} μmol NADH oxidised to NAD$^+$ per mol LDH per min) is also in excellent agreement with the relative rate calculated from the activation parameters. The bluefin tuna data are from ref. 23. The rabbit data (obtained using a phosphate buffer system) are from ref. 9.

achieved through maintenance of low enzyme concentrations[5]. The alternative explanation we propose to account for the high activation parameters of enzymes from high-pressure-adapted organisms is based on an analogy between high temperature and high pressure effects on enzymes. For enzymes of high body temperature species like mammals, a concomitant of low catalytic efficiency (Table 1) is a high degree of structural stability; for example, a high resistance to thermal denaturation[8,11,12]. This relationship has been found for LDHs, and has been interpreted in terms of additional weak bonds, for example, hydrogen bonds, stabilising the mammalian enzyme–cofactor–substrate ternary complex relative to the complex formed with a fish LDH[11]. Elevated hydrostatic pressures may also favour selection for more structurally stable enzymes which, like the enzymes of high body temperature species, also have reduced catalytic efficiencies. In fact, the M_4-LDHs of the four deep-sea fishes have efficiencies similar to that of a mammalian M_4-LDH (Table 1).

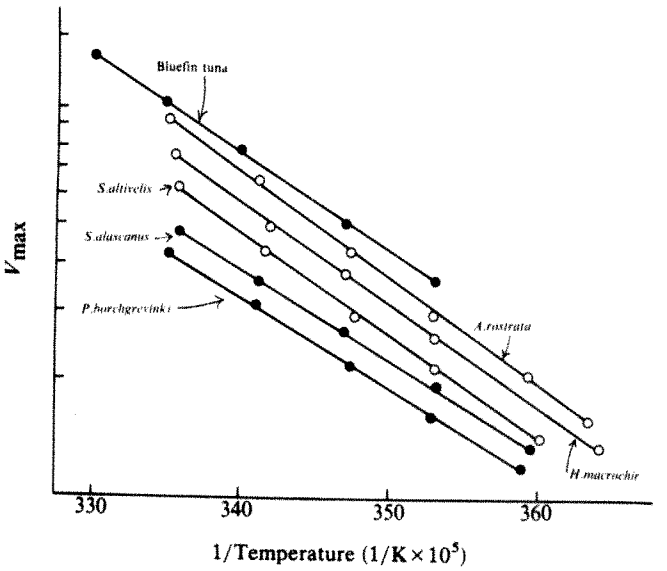


Fig. 1 Arrhenius plots (log V_{max} against reciprocal of absolute temperature (K)) for M_4-lactate dehydrogenase reactions of several deep-sea and shallow-living teleost fishes. The V_{max} values were determined using M_4-isozymes of LDH purified by oxamate affinity chromatography. Purification details are given in ref. 7. LDH activity was assayed by following the decrease in absorbance at 340 nm due to oxidation of NADH in a thermostatted spectrophotometer. Cuvette temperatures were held within ±0.2 °C of the stated temperatures. The assay medium contained 80 mM imidazole/HCl buffer, adjusted to *p*H 6.98 at 20 °C. At all of the other experimental temperatures, the *p*H of this imidazole buffer system follows the intracellular *p*H of ectothermic vertebrates (see ref. 8). The NADH concentration was 150 μM. At each temperature 6–9 pyruvate concentrations were used to determine theoretical V_{max}. Data were analysed using the weighted linear regression technique of Wilkinson[19]. Note that the plots are positioned on the axes to show differences in slope, so the V_{max} values do not reflect the actual differences in substrate turnover number (see Table 1) of the LDHs of the shallow-living (●) and deep-living (○) fishes.

The differences in kinetic properties between M_4-LDHs of shallow- and deep-living species suggest that enzymatic adaptations may be important in establishing bathymetric distribution patterns (although biological interactions must also have an important role). Pressure-sensitive K_m values of LDHs of shallow-living fishes may make these enzymes unsuitable for function at high and/or variable pressures, and thus might help restrict the depth distributions of these species[6,7,21]. On the other hand, even though the M_4-LDHs of deep-living fishes are very insensitive to pressure and would seem on this basis to be able to function at both shallow and great depths, the catalytic inefficiencies of these enzymes may place them at a significant disadvantage at low pressures. To possess the same glycolytic capacity as a shallow-water fish, a deep-sea fish would have to maintain a high -concentration of LDH molecules in its cells, a demand which is at odds with the low protein concentrations of the muscle fluids of these species[5,7,16,17].

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Post-transcriptional control in the early mouse embryo

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The earliest stages of mouse embryogenesis, from fertilisation to the two-cell stage, are characterised by an extremely low level of RNA synthesis. Indeed, during this period, RNA polymerase II activity[1] and incorporation of labelled precursors into heterogeneous RNA[2] are not detectable, and there is no increase in the poly(A) content of the embryo, but rather a slight decrease[3]. The rate of protein synthesis remains low and relatively constant throughout the one- and two-cell stages[4]. However, qualitative analysis of the protein synthetic profile on SDS gels has revealed changes which appear around the late one-cell to early two-cell stage[4–6]. This early change in the pattern of polypeptide synthesis represents the first major qualitative molecular change found so far in development. We present evidence which suggests that the increased synthesis at the early two-cell stage of a small number of polypeptides of molecular weight 35,000 is not dependent on transcription, but rather represents control at a post-transcriptional level using mRNAs synthesised before fertilisation.

Figure 1*a* shows the pattern obtained when newly fertilised embryos were labelled for 3 h with ^{35}S-methionine and the radioactive polypeptides separated on a two-dimensional gel of the O'Farrell type[7]. Although there are a few notable differences between this and the pattern obtained from unfertilised eggs (Fig. 2*a*) the patterns are remarkably similar. The corresponding pattern from early two-cell embryos is shown in

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Fig. 1*b*. It is clear that most of the polypeptides that are made immediately after fertilisation continue to be synthesised at the two-cell stage. In addition, among the changes which can be observed at least three polypeptides (arrowed) with molecular weights around 35,000 are made in greatly increased amounts at the later time.

If this increase was due to the *de novo* synthesis of mRNA, it should be possible to block the changes by adding a specific inhibitor of transcription. A suitable inhibitor is α-amanitin, which at levels of 10–100 $\mu g\,ml^{-1}$ specifically blocks mRNA synthesis, inhibits RNA polymerase II[1,8–10] and has been shown to be effective at these doses in early mouse embryos[11,12]. The presence of 11 $\mu g\,ml^{-1}$ α-amanitin in the culture medium did not prevent the first cell division, and Fig. 1*c* shows that it also did not block the appearance of the characteristic early two-cell polypeptides. This result was found in four separate experiments and also when 100 $\mu g\,ml^{-1}$ α-amanitin was used (data not shown). However, before it can be concluded that control of the synthesis of the three polypeptides is at a post-transcriptional level, it is necessary to demonstrate that the inhibitor actually enters the cells.

As the incorporation of radioactive precursors into mRNA is undetectable over the period being studied, an indirect approach had to be adopted. It has been widely reported that α-amanitin can arrest or markedly retard cleavage in preimplantation mouse embryos[11,13–15]. Since α-amanitin is an irreversible inhibitor[16], a brief early pulse of the drug at the one-cell stage should produce effects at a later stage of development when transcription is required. As one-cell embryos from CFLP mice do not readily develop *in vitro* beyond the two-cell stage, embryos from B10 × CBA F_1 hybrids (courtesy of Dr M. H. Kaufman) were used. Table 1 shows the results from such an experiment in which newly fertilised eggs were pulsed with 11 $\mu g\,ml^{-1}$ α-amanitin for 6 h before they were transferred to fresh medium free of α-amanitin and allowed to develop further *in vitro*. None of the α-amanitin-treated embryos progressed beyond the two-cell stage, whereas 82% of the control embryos which reached the two-cell stage developed to the three- or four-cell stage within 56 h of fertilisation.

From this we conclude that α-amanitin does enter one-cell embryos, and that transcription by RNA polymerase II is not required for the first cell division. We also conclude from Fig. 1 that most of the mRNAs present at fertilisation are still active some 24 h later, at the early two-cell stage, and that the appearance of the new polypeptides at this stage is not due to synthesis of new mRNAs by RNA polymerase II. The changes in polypeptide synthetic profile probably therefore represent either post-transcriptional control or α-amanitin resistant transcription, as for example from a viral or mitochondrial genome.

To distinguish transcriptional from post-transcriptional control we investigated whether mRNAs coding for the 35,000-molecular weight polypeptides were present (but not translated) in unfertilised eggs. Total RNA was extracted from about 200

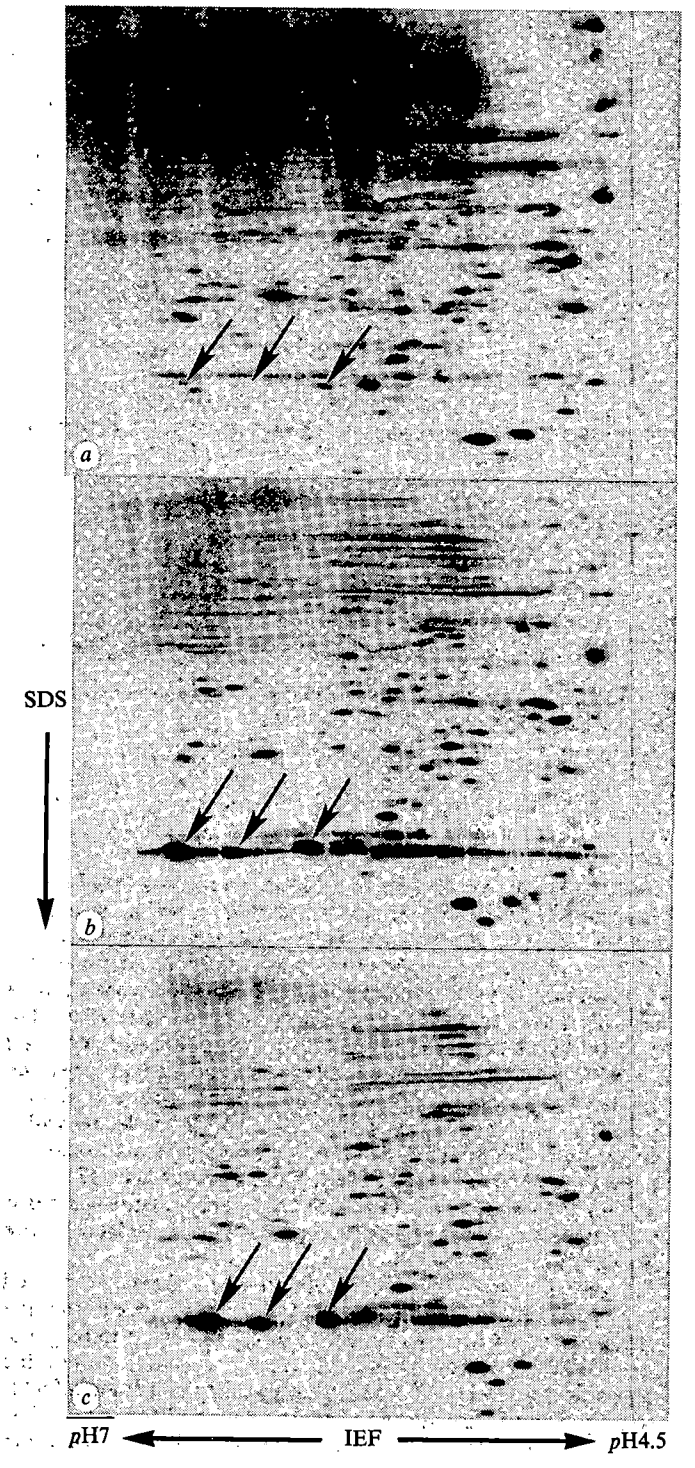


Fig. 1 Two-dimensional fluorographs of ^{35}S-methionine-labelled polypeptides from fertilised eggs (*a*), two-cell embryos having been cultured for 24 h *in vitro* from the one-cell stage (*b*), and two-cell embryos having been cultured for 24 h *in vitro* from the one-cell stage in the presence of 11 $\mu g\,ml^{-1}$ α-amanitin (*c*). Cumulus masses were recovered at 14 h after HCG from the oviducts of superovulated female CFLP mice which had been mated with CFLP males. The cumulus cells were removed by brief treatment in hyaluronidase (1 $mg\,ml^{-1}$), and were then washed through several changes of phosphate-buffered medium (PBM) containing 10% fetal calf serum[20]. The pooled embryos were divided randomly into three groups; dead or disorganised embryos being excluded. One group was incubated for 3 h in 100 μl of medium 16 + 4 $mg\,ml^{-1}$ bovine serum albumin[21] with 10 μl of ^{35}S-methionine (~5 $mCi\,ml^{-1}$). At the end of this period 30 unfertilised eggs were transferred to 20 μl lysis buffer and stored at −70 °C. The presence of two pronuclei and a second polar body was taken as evidence of fertilisation. The remaining two groups were incubated for 24 h in 100 μl drops of medium 16 + BSA in the presence or absence of 11 $\mu g\,ml^{-1}$ α-amanitin. At the end of the culture period the embryos were scored for stage of development and 10 μl of ^{35}S-methionine was added to each drop. After a further 3 h the two-cell embryos in each group were collected in 20 μl of lysis buffer. All incubations were performed at 37 °C in an atmosphere of 5% CO_2 in air. Two-dimensional polyacrylamide electrophoresis in denaturing conditions was carried out over a *p*H range of 4.5 to 7 for the first dimension and an SDS gradient of 7–15% acrylamide for the second as described previously[13,22]. The gels were impregnated with 2,5-diphenyloxazole[23] and exposed to preflashed[24] Fuji Rx X-ray film for between 2 and 6 weeks. Analysis of the film was performed as described previously[13]. The arrows indicate polypeptides weakly labelled in the fertilised egg but strongly labelled at the two-cell stage both in the presence or absence of α-amanitin.

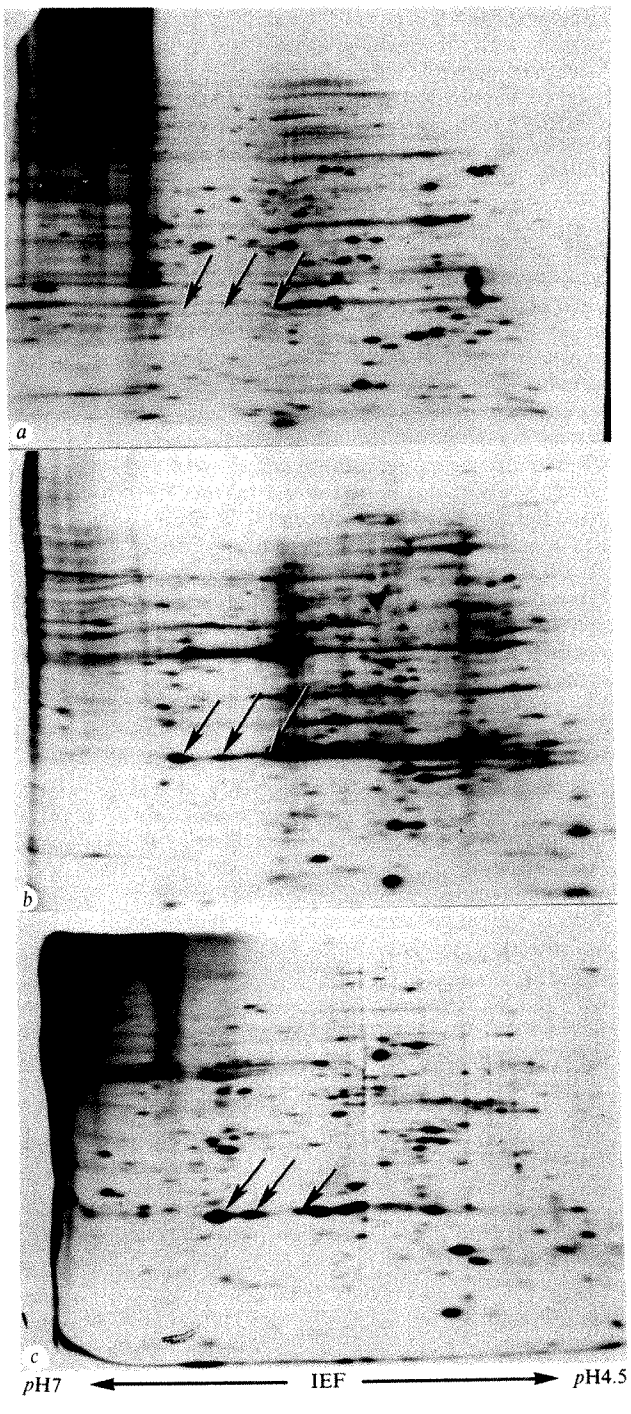


Fig. 2 Two-dimensional fluorographs of ^{35}S-methionine-labelled polypeptides from 60 intact unfertilised eggs (*a*), mRNA from 200 unfertilised eggs translated *in vitro* (*b*), and from 50 intact early two-cell embryos (*c*). Cumulus masses were recovered at 15 h after HCG from the oviducts of superovulated female CFLP mice. The cumulus cells were removed (as for Fig. 1), and 60 unfertilised eggs were labelled for 3 h in medium containing ^{35}S-methionine (as for Fig. 1). mRNA from 200 eggs was extracted and translated in a specially modified message-dependent reticulocyte lysate system as described previously[17]. Two-cell embryos were flushed from the oviducts of superovulated mice 40 h after HCG and labelled as for the unfertilised eggs. Two-dimensional electrophoresis and processing was as for Fig. 1. The arrows show polypeptides which are not strongly labelled until the two-cell stage but are translated *in vitro* from egg mRNA.

Table 1 Development of B10×CBA 1-cell embryos* in the presence of 11 μg ml^{-1} α-amanitin for 6 h

Stage	−α-amanitin		+α-amanitin	
	48 h post HCG	68 h post HCG	48 h post HCG	68 h post HCG
1-cell	17	10	18	14
2-cell	23	5	25	29
3-cell	0	3	0	0
4-cell	0	20	0	0
Dead/disorganised	2	4	2	2
Total	42	42	45	45

Embryos were washed in medium free of α-amanitin at 24 h post HCG and examined at 48 and 68 h post HCG.
* 18 h after HCG administration.

unfertilised eggs and translated in the mRNA-dependent reticulocyte lysate system as described previously[17]. Figure 2 shows a comparison between the products obtained by *in vitro* translation and the polypeptides labelled in either intact unfertilised eggs or early two-cell embryos. The two-cell marker proteins (arrowed) were prominent amongst the cell-free products, even though they were virtually undetectable in the unfertilised egg pattern. This result was consistently found in eight experiments. It could perhaps be argued that the cell-free system might selectively translate the mRNAs for these polypeptides. However, preliminary experiments indicate that these polypeptides are no more prominent among the translation products of RNA from two-cell embryos (data not shown).

Thus, there are a few polypeptides which are prominent products of the early two-cell mouse embryos but which are only very minor products of unfertilised or newly-fertilised eggs. The appearance of these polypeptides is not blocked by the transcriptional inhibitor α-amanitin, and mRNAs coding for them are present in unfertilised eggs. Although we cannot absolutely exclude the possibility of a rapid transcriptional event occurring at fertilisation before the eggs can be transferred into the α-amanitin it seems reasonable to conclude that the increased synthesis of these polypeptides is under post-transcriptional control[18]. Several mechanisms for this type of control could operate[19]. The changes in the two-dimensional gel pattern cannot be explained merely by alteration in the charge of the polypeptides, as their appearance is also detectable on one-dimensional SDS gels. However, we cannot exclude the possibility of very rapid degradation of the polypeptides in the egg but not at the two-cell stage. Physical sequestration of the corresponding mRNAs could also explain the results, but the basis for such masking is presently unclear. Only a few polypeptides are involved, and their close proximity on two-dimensional gels suggests that they may be structurally related and under co-ordinate control. It seems that the developing embryo requires large amounts of these proteins at a time when a sudden burst of transcription is inconvenient or impossible, and therefore relies on a post-transcriptional control mechanism. In contrast, development beyond the two-cell stage seems to require some transcription of the embryonic genome (Table 1)[13].

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Myosin isoenzyme redistribution in chronic heart overload

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Since the first observation by Spann *et al.*[1], it has become clear that in cardiac hypertrophy induced by a mechanical overloading, the velocity of shortening of the cardiac muscle (V_{max}) is reduced (see ref. 2 for review). Most authors agree that this mechanical alteration is accompanied by a decrease in the Ca^{2+}-dependent ATPase activity of myosin (see ref. 3 for review). The molecular basis of such changes was unknown because the structural modifications of the myosin molecule were ill-defined[4–6]. Nevertheless, it has recently been shown that, like skeletal muscle myosin[7], cardiac myosin is composed of several polymorphic forms, comparable to isoenzymes[8,9]. In the skeletal muscle, new functional requirements can induce changes in both contractile activity and type of myosin isoenzyme synthesised[10–13]. We now report that an increase in cardiac work produced by mechanical overloading in rats induces the preferential synthesis of a cardiac myosin isoenzyme characterised by specific immunological and electrophoretic properties and exhibiting a lower ATPase activity. This adaptive change could account for the reduced shortening speed of this hypertrophied cardiac muscle.

Cardiac hypertrophy was induced in male Wistar rats (200 g, Animalabo) either by a volume[14] or pressure overload[15], or by combining both procedures[14], that is, abdominal aorta constriction and induction of aortic incompetence 2 weeks later (see Table 1 legend). Animals exhibiting 60 to 100% cardiac hypertrophy by any of the above procedures were selected for this study, but it should be pointed out that the most reproducible results were obtained with the two-step procedure. Enzymatic, immunological and electrophoretic tests were performed on myosins from the same hypertrophied hearts and from hearts of sham-operated animals.

Steady-state Ca^{2+}- or Mg^{2+}-dependent ATPase activities of myosins from hypertrophied hearts were both significantly depressed, whereas the K^+-activated ATPase remained unchanged (Table 1). This is in full agreement with earlier observations in rats[16,17] and in other animal species[4,18–20]. Since it has often been suggested that this diminished Ca^{2+}-dependent activity might be due to artifactual denaturation, the functional integrity of individual myosin preparations was assessed by measuring their early phosphate burst size, that is the number of ATP moles cleaved per myosin molecule. As shown in Table 1, the myosins extracted from both hypertrophied and normal hearts cleaved 0.8 mol of ATP per active site. This value, in close agreement with previously published results for normal skeletal and cardiac muscles[23–25], rules out the possible presence of denatured, aggregated or oxidised molecules[23,25]. Such absence is consistent with the functional integrity of the site known as SH1 in the hypertrophied rabbit myocardium[20]. In addition, electrophoresis on SDS-polyacrylamide gels visualised the heavy subunits as single bands (not shown) indicating that the degraded components observed by others in dogs[6] were not present in our preparations. The decrease in ATPase specific activity was therefore not due to molecular denaturation, but more likely indicated the presence of a new myosin species with different enzymatic activities.

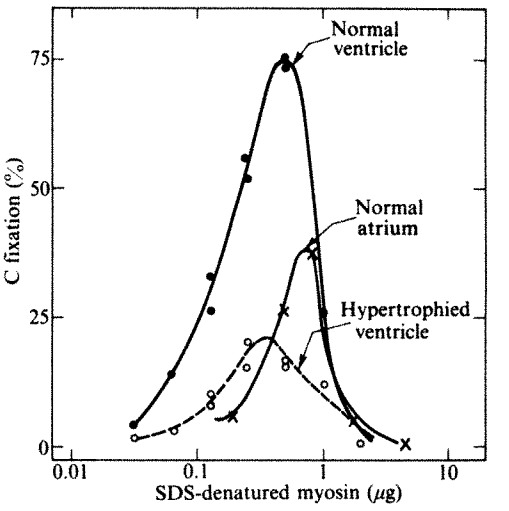


Fig. 1 Microcomplement fixations with antiserum 47-3-70 to normal rat ventricular SDS-heavy meromyosin. Antiserum dilution 1:5,250. SDS-denatured myosins were obtained from the ventricles and atria of sham-operated animals and the ventricles of a hypertrophied rat heart (100% hypertrophy preparation no. 5 in Table 1). Antibodies were raised in guinea pigs against purified rat ventricular heavy meromyosin[28], denatured by heating for 5 min at 90 °C in the presence of SDS (5 mg per mg protein), 2% 2-mercaptoethanol, 50 mM NaCl and 100 mM Tris-HCl, *p*H 7.6. Excess SDS was removed immediately before immunisation by gel filtration on a 2 × 15 cm Sephadex G-10 column[27]. Microcomplement fixations were produced according to Levine[26], in a final volume of 0.7 ml. Before the assays, myosins were denatured and excess SDS was removed as described above. The mean difference between two controls was equal to 4.8%, s.e.m. = 1.7, $n = 7$. These antibodies are specific to a denatured configuration of heavy-meromyosin and myosin induced by treatment with either guanidine or SDS[27] and, as shown here, can easily discriminate between atrial and ventricular myosin isoenzymes.

This possibility was first tested by an immunochemical approach, using micro-complement fixation (MCF), a technique known for its ability to detect small structural changes[26]. Antibodies specific to a denatured structure of rat ventricular myosin were obtained by injecting guinea pigs with heavy meromyosin denatured by SDS treatment[27]. SDS-denatured myosin from each hypertrophied heart was made to react with antisera from two different animals. There was a vertical shift in the maximum amount of complement fixed (15–35%), compared to the controls (50–70%) (Fig. 1). The immunological distances between normal and hypertrophied heart myosins were calculated by using several dilutions of antisera for the reactions[29]. Distances were between 10 and 30, the maximum value corresponding to the myosin with the lowest enzymatic

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Table 1 Enzymatic analysis of myosins from normal and sham-operated rat hearts (S) and hypertrophied hearts (H)

Type of overloading, interval between surgery and killing (% of heart hypertrophy)	Burst size (mol ATP per myosin site)		Specific activity in steady state (nmol P_i mg^{-1} min^{-1})					
			Ca^{2+}-activated		Mg^{2+}-activated		K^+-activated	
	S	H	S	H	S	H	S	H
(1) Aortic incompetence, 130 days (60%)	0.85	0.80	1,210	760	—	18	610	540
(2) Aortic incompetence, 141 days (93%)	0.85	0.85	1,180	860	37	27	660	690
(3) Aortic stenosis, 35 days (100%)	0.75	0.70	1,190	880	43	31	620	720
(4) Aortic stenosis, 10 days, +incompetence 92 days (90%)	0.85	0.80	1,020	730	27	18	620	760
(5) Aortic stenosis, 10 days, + incompetence 63 days (97%)	—	—	980	540	29	12	710	750

50% abdominal aorta constriction was obtained using the method of Cutilletta *et al.*[15]. Aortic incompetence was produced by forcing a stiff polyethylene catheter (no. 3 to 4 Biotrol) through the left carotid and then the aortic cusps. At various times after surgery, rats were killed by a blow on the head and hearts were rapidly excised. Ventricles were detached from the atria and bases of the great vessels, weighed (Vw) and frozen in liquid nitrogen within 2 min. For each animal, theoretical ventricular weight (Thw) was calculated according to the following regression curve, established in 152 controls: Thw (g) = $[(2.02 \times \text{body wt}) + 197] \times 10^{-3}$. The per cent hypertrophy was equal to $(Vw - Thw) \times 100/Thw$. Myosin was prepared essentially according to Offer *et al.*[21], except that ammonium sulphate fractionation was performed in the presence of 100 mM sodium pyrophosphate, 4 mM ATP and 2 mM dithiothreitol *p*H 7.0. The A_{280}/A_{260} ratio was between 1.4 and 1.6. Early phosphate burst size was determined manually by a slightly modified version of the method of Taylor and Weeds[23], in stoichiometric conditions[24], in which 10 μM myosin site (5 μM myosin) and 10 μM radioactive ATP were mixed at 22 °C, in a medium consisting of 0.5 M KCl, 50 mM Tris-HCl, 5 mM $MgCl_2$ and 0.1 mM dithiothreitol, *p*H 7.4. Steady state activities were measured at 25 °C in the presence of 2.5 mM ATP, 50 mM Tris-HCl, *p*H 7.5 and either 5 mM $CaCl_2$, 5 mM $MgCl_2$ or 1 M KCl and 5 mM EDTA. Inorganic phosphate was determined colorimetrically with malachite green[22].

activity. The present results confirm and extend our recent observation of an antigenic difference between native myosins from normal and hypertrophied rat hearts[17]. It is thus unlikely that such immunological changes reflect conformational changes only. Indeed numerous observations have shown that vertical shifts of the micro-complement fixation curves indicate amino acid substitutions[29].

Finally, the presence of different myosin isoenzymes in the normal and hypertrophic hearts was screened by electrophoresis of the native proteins in a non-dissociating medium. We used this technique according either to Hoh *et al.*[9], or to a modified version of a previously described method[30,31]. In agreement with the findings of Hoh *et al.*[9], we observed in a highly reproducible manner three isoenzymes V_1, V_2 and V_3 (Fig. 2) in normal adult rat ventricles. V_1 had the highest mobility and was predominant in both right and left ventricles. The electrophoretic pattern was different in the ventricles from hyperthrophied hearts (Fig. 2); making the reasonable assumption that the three isoenzymes were the same as in the normal hearts, a redistribution of V_1, V_2 and V_3 was found, with a predominance of V_3 in the largest and an intermediary pattern in the less affected hearts. Furthermore, the isoenzymic shift was more pronounced in the left ventricle (not shown) which is more affected in our experimental model. According to Hoh *et al.*[9] and to our unpublished results, the specific ATPase activity of V_3 is lower than that of V_1; the increase in V_3 may therefore account for the drop in overall enzymatic activity (Table 1). These findings are consistent with the isoenzymic switches already reported between V_1 and V_3 (ref. 9). As in our model of hypertrophy, hypophysectomy induces an increase in V_3 and a fall in myosin ATPase activity,

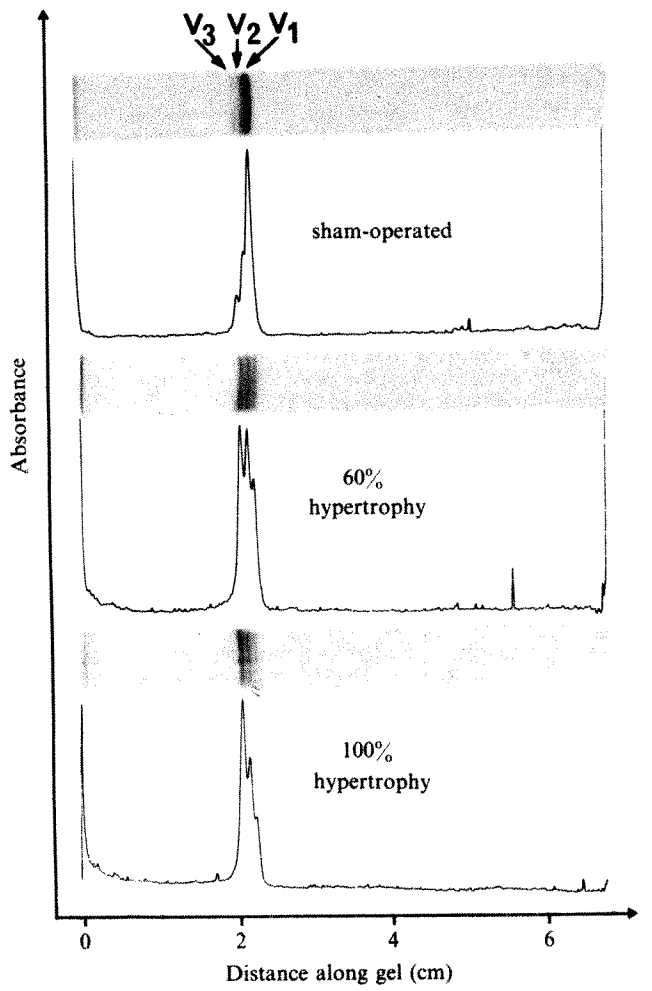


Fig. 2 Typical electrophoretic patterns and densitometric profiles for native myosins from ventricles of one normal (sham-operated) and two hypertrophied hearts (60 and 100% hypertrophy respectively). Myosin samples (1 to 3 μg) were run for 16 h on cylindrical gels (60 × 5 mm), 3.88% in acrylamide and 0.12% in *N-N'*-methylene bis-acrylamide at 14 V cm^{-1} and 2 °C, in 20 mM sodium pyrophosphate (*p*H 8.8) in the presence of 10% glycerol[9]. V_1, V_2 and V_3 correspond to the three components described by Hoh *et al.*[9]. Identical patterns were observed when crude tissue extracts, prepared either with Guba-Straub's buffer or according to Hoh *et al.* where run instead of purified myosins. When run on a second pyrophosphate gel, the main isoenzyme, V_1 in the normal heart and V_3 in the hypertrophied one, migrated as a single band. The electrophoretic pattern was independant of the acrylamide concentration (2.8 to 4%). The same results were obtained in a slightly different medium consisting of 40 mM sodium pyrophosphate (*p*H 8.5), 1 mM EDTA and 0.01% 2-mercaptoethanol as described by d'Albis *et al.*[31].

whereas subsequent thyroxine treatment produces both an increase of V_1 and a rise in the enzymatic activity.

It is now accepted that there is a close correlation in different types of skeletal muscles[32] and in hearts of different animal species[33] between contractile element shortening capacity and myosin ATPase. Since the velocity of shortening of the cardiac muscle is reduced in gross cardiac hypertrophy induced by a mechanical overload (reviewed in ref. 2), the predominance we demonstrate in this muscle of a myosin isoenzyme of lower activity is not surprising. On the contrary thyroid hormone increases cardiac performance and more especially V_{max} (reviewed in ref. 34) and induces in hypophysectomised rats[9] as well as in euthyroid rabbits[35], the synthesis of an isoenzyme of higher enzymatic activity. All these findings suggest that contractile activity of the diseased myocardium is controlled as in normal skeletal and cardiac muscles[32,33], by the rate of hydrolysis of ATP by its myosin isoenzyme. In addition, adaptation in cardiac performance seems to be mediated by a change in myosin species, a process which can be related to the adaptive responses of the skeletal muscles to new functional requirements[10–13]. The normal peak active isometric tension observed in cardiac muscles after a pressure overload[36] suggests that such hypertrophied muscles, in spite of their contractile defect, work with normal efficiency. Similarly, slow skeletal muscles work more efficiently when contracting at slow speeds, compared to fast muscles, and this characteristic also seems to be connected with decreased ATPase activity[37]. The molecular basis of adaptation in cardiac performance we describe could therefore account for the compensatory step observed in chronic heart overload. It might be also hypothesised that cardiac insufficiency occurs when the adaptive change has reached its limit, that is, when the isoenzymic shift is a maximum.

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Three-dimensional structure of hyper-modified nucleoside Q located in the wobbling position of tRNA

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The hyper-modified nucleoside Q (queuosine) is exclusively located in the wobbling position of anticodons of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ that recognise codons NA^{U}_{C} (ref. 1). Queuosine and its hexose-containing derivatives[2,3] are widely distributed in microorganisms, animals and plants[4,5]. We confirm here the chemical structure of queuosine as 7-(3, 4-*trans*-4, 5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine[2,6] (Fig. 1). The unique structural features of Q are the unusual cyclopentenediol side chain and the 7-deazaguanine ring. Queuosine is one of the most complicated modified nucleosides found thus far[7].

For elucidating the biological significance of this complicated modification, it is important to determine the conformation of the Q unit in the tertiary structure of tRNAs: is the conformation of the anticodon loop very different from that of usual tRNAs, such as yeast $tRNA^{Phe}$ (refs 8–10), because of the existence of the bulky side group? Does the side chain approach the anticodon–codon interaction site and thus influence the codon recognition properties? Is the side chain buried in the anticodon loop, or exposed enough to be recognised by another molecule? To answer these questions, we first determined the three-dimensional structure of queuosine 5′-monophosphate (pQ) by X-ray crystallography. We then incorporated this conformation of Q into the anticodon of tRNA, on the basis of the

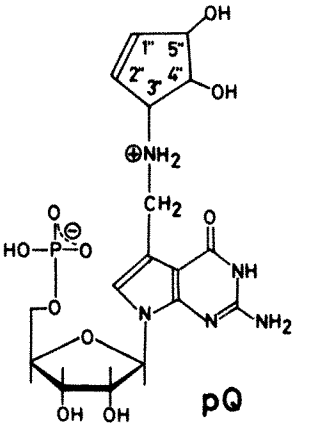


Fig. 1 Structural formula of pQ. Crystal data: 7-(3, 4-*trans*-4, 5 - *cis* - dihydroxy - 1 - cyclopenten - 3- ylaminomethyl) - 7 - deazaguanosine 5′-monophosphate (queuosine 5′-monophosphate, pQ), $C_{17}H_{14}D_{10}N_5O_{10}P \cdot 7/2D_2O$, $FW = 569$, orthorhombic, space group $P2_12_12_1$, $Z = 8$, $Dx = 1.528\ gcm^{-3}$, $a = 26.895(5)$, $b = 7.707(2)$, $c = 23.883\ (10)$ Å, $V = 4{,}951$ Å^3

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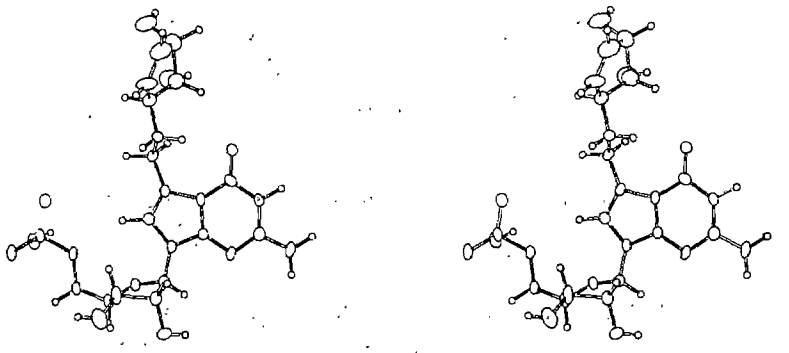


Fig. 2 Stereo view of pQ (molecule I) in crystalline form.

coordinates of yeast $tRNA^{Phe}$ (ref. 11), as done previously for some other modified nucleosides[12–14]. The results indicate that the bulky 7-substituent group of Q takes the planar *trans* conformation and is fully extended outwards, so that it does not interfere with codon–anticodon interaction. Thus it seems possible that the cyclopentenediol side chain of Q may interact with other molecules in some process other than protein biosynthesis.

Queuosine 5′-monophosphate was isolated from a nuclease P_1 digest of *Escherichia coli* tRNA by Dowex 1 column chromatography[15]. pQ in the free acid form was crystallised from concentrated D_2O solution (pD, 4.5), so that the phosphate group was monoanionic and the aminomethyl group at position 7 of the 7-deazaguanine ring was positively charged. A small fragment of $0.3\times0.08\times1.5$ mm was cut from a large crystal of $0.3\times0.08\times3$ mm and sealed in a thin-walled glass capillary to prevent loss of water of crystallisation. The specimen was mounted on a Philips four-circle diffractometer and the unit cell dimensions and intensity data were measured with monochromated CuKα radiations using a graphite plate. The crystal data are given in Fig. 1 legend. The intensities were measured within a 2θ range of 6–120° by the θ–2θ scan method. A total of 5,589 reflections were measured as above the $I>2\sigma(I)$ level, including 965 symmetry equivalent reflections ($\bar{h}kl$ versus hkl). Deterioration of the crystal was monitored by measuring the intensities of three standard reflections. The sums of the three intensity values measured at the beginning and at the end of the measurement showed only a slight decrease (1.4%) throughout the period of measurement.

The crystal structure was solved by the direct method based on the procedure using MULTAN[16]. An E map calculated for the most probable set gave a plausible arrangement of peaks for phosphate groups. The whole structure was established by successive use of difference Fourier and least-squares methods. At the final stage of the refinement, water oxygen atoms were located on the difference electron density map. Four water molecules were found to occupy several sites. Their positions were assigned so that each of the four water oxygen atoms occupy with a half occupancy factor at the two separate positions not far more than 2 Å. A block-diagonal least-squared refinement for 693 parameters of 77 atoms yielded an R factor of 0.117 for 4,624 observed reflections. Hydrogen atoms were not included in the refinement but they were located by model building.

The present study confirms the chemical structure of queuosine reported earlier[2,6]. The geometries of the phosphate and imino groups indicated that the phosphate group bears one negative charge and the imino group in the side chain is protonated. Thus the molecule takes the dipolar form. The absolute configuration was determined assuming that the ribose group takes the D-configuration. It was then confirmed that the absolute configuration at C(3″) of the cyclopentene ring is the same as that determined by synthetic and CD and ORD studies[17]. There are two crystallographically independent molecules (I and II), which have quite similar molecular structures; the r.m.s. differences between the two molecules are 0.032 Å and 1.6° for bond lengths and bond angles, respectively, and the dihedral angles are nearly equal, being within 7° of each other. The bond lengths and bond angles of the pyrimidine moiety of the 7-deazaguanine ring are almost equal to those of the unmodified guanine ring[18]. It is likely, therefore, that the hydrogen-bonding or base-pairing properties of the Q base (queuine) are little influenced by replacement of the nitrogen atom by a carbon atom at position 7. The ribose ring takes the C(3′)-endo form, with the exocyclic C(4′)—C(5′)—O(5′) bond in the g^+-t form ($\psi=52°$ and 52° and $\phi=-171°$ and −174°, for molecules I and II, respectively) and the glycosyl bond in the anti form ($\chi=27°$ and 30°, respectively). The side chain at position 7 of the 7-deazaguanine ring is fully extended; C(7)—CH_2—NH_2^+—C(3″)—C(2″) takes the planar *trans* conformation. An intramolecular hydrogen bond is formed between the protonated imino group and O(6) of the 7-deazaguanine ring. The dihedral angle of C(8)—C(7)—CH_2—NH_2^+ is expected to be about 90°, as is commonly observed in related structures having a methylenic chain linked to the planar ring system. Actually however, this dihedral angle of pQ is twisted to as much as about 120°, possibly by formation of a rather strong intramolecular hydrogen bond NH_2^+ ... O(6). The bulky 7-substituent group is extended (Fig. 2), but not towards the hydrogen bonding moiety of the Q base

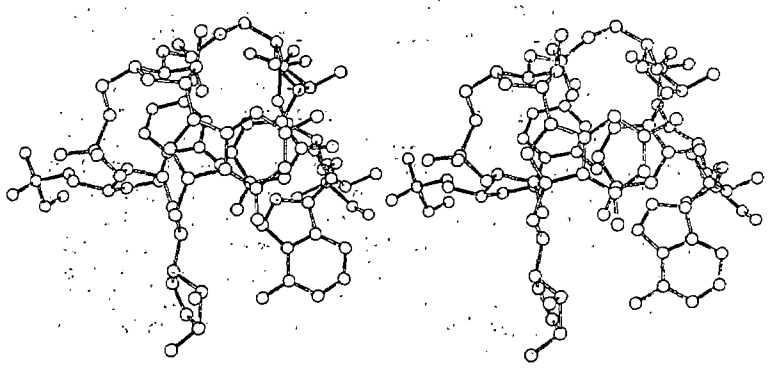


Fig. 3 Stereo view of U-Q-U-A, as constructed from the atomic coordinates of pQ (the present study) and yeast $tRNA^{Phe}$ (ref. 11).

and the third letter of the codon (C or U). This also indicates, in accord with biochemical observations, that the codon recognition properties of Q are not significantly different from those of G (ref. 1 and S. Noguchi and S.N., unpublished). The cyclopentene ring is puckered; C(3″)—C(2″)=C(1″)—C(5″) is coplanar but C(4″) is displaced by 0.5 Å from this plane on the same side as the NH_2^+ group, possibly due to the existence of a diol {O(4″)H and O(5″)H} in the *cis* configuration. This puckered form is stabilised by the equatorial orientation of the large substituent (NH_2^+—CH_2—···).

The atomic coordinates of yeast $tRNA^{Phe}$ (ref. 11) were used for the model-building study on the spatial arrangement of the modified nucleoside Q in the anticodon loop of tRNA. The first letter of the anticodon, Gm(34), was replaced by Q with the conformation found in the present study on the crystal of pQ. The second letter of the anticodon, adenine(35), was replaced by uracil, but the third letter, adenine(36), was retained. This sequence QUA corresponds to that in the anticodon of the $tRNA^{Tyr}$ of *E. coli*. Figure 3 shows a stereoscopic view of the three-dimensional structure involving U(33)—Q(34)—U(35)—A(36). It may be seen that Q is now incorporated into the first position of the anticodon without any steric repulsion from other parts of the anticodon loop. The cyclopentenediol group of Q is exposed out of the anticodon, in contrast to the short group of 2-thiouridine derivatives, which is more or less buried in the anticodon loop[13,14].

In addition to the intra-unit hydrogen bond with O(6), the NH_2^+ group of Q may also be involved in a hydrogen bonding interaction with ribose O(2′) of the preceding U(33); the distance between the imino nitrogen and ribose oxygen is as short as 3 Å and N—H···O is nearly linear. The side group of Q(34) is thus fixed by these two hydrogen bonds, and the *cis*-diol group is orientated out to the open space, away from the base-pairing moiety of the anticodon.

The biochemical function of Q in protein biosynthesis is not yet understood. Q has a wobbling property not significantly different from that of G. Extensive studies on the properties of $tRNA^{Tyr}$ containing G in place of Q, isolated from Q-deficient mutants of *E. coli* have not revealed any differences between Q and G with respect to binding of $tRNA^{Tyr}$ to ribosomes or aminoacylation (S. Noguchi and S. N., unpublished). Two species of rabbit reticulocyte $tRNA^{His}$ containing either Q or G were used with equal efficiency in haemoglobin biosynthesis[19]. These observations are consistent with the conformation of Q found in the present study by X-ray diffraction analysis. This hypermodified nucleoside Q is widely found in tRNAs of various organisms and thus is expected to be essential for some biological process other than protein biosynthesis. Q in *Drosophila* tRNA is suggested to have a role in cell differentiation or expression of a particular enzymatic activity[5,20]. In tumour cells, under-modified tRNA containing guanosine in place of Q appears specifically[21].

In conclusion, the conformational characteristics of the modified nucleoside Q appear to bring the cyclopentenediol group out of the anticodon loop, where it does not interfere with recognition of the codon of mRNA. This bulky side chain with its unique structure, charge and reactive group constitutes a suitable site for specific interaction with protein or other components. Thus the exposed cyclopentene ring (and orientation of the *cis*-diol group) of Q is possibly important for some biochemical function of tRNA other than protein biosynthesis.

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Antiparallel and parallel β-strands differ in amino acid residue preferences

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A β-strand is a particular type of extended sequence of amino acid residues, an element of secondary structure of proteins. β-sheets are an assembly of strands, often bringing together parts of the protein which are separated along the backbone. As such, β-sheets are an element of tertiary structure. Parallel (β_P) and antiparallel (β_A) arrangements of strands in a sheet differ in the hydrogen bond pattern between strands, as shown schematically in Fig. 1, and in the type of chain connectivity they allow: short reverse turn connections for β_A and longer crossover connections for β_P (refs 1–3). Most present secondary structure prediction methods (for reviews refs 4–6) use a four-state distinction of secondary structure: α-helix, β-strand or extended, reverse turn, and 'random coil' (everything else). With a data base of 30–40 different protein structures, the conformational preferences for all amino acid residues in these four states seem to have converged[7]. However, the steadily increasing data base of structurally known proteins makes a refinement of the four-state description feasible. Although more refined classifications of conformational states based on finer subdivisions of (φ, ψ)-space have been made[8,9], we prefer making distinctions based on structural environment. Using a novel definition of β-sheet structure in terms of the tertiary structure juxtaposition of strands, we have analysed residue contacts in known β-sheets and report here secondary structure preferences for the 20 amino acids, separately for antiparallel and parallel arrangements of strands. The distinction between the two arrangements results in strikingly different and sharpened sets of preference parameters, including some of the largest values reported so far for any substructure. These results point the way towards a basic improvement of secondary structure predictions by further distinction of secondary structure elements according to tertiary structure environment. Beyond secondary structure prediction, the different preferences for β_A and β_P may aid in predicting the tertiary interaction between strands.

Our independent compilation of β_A- and β_P-residues in 30 proteins (a list of the proteins is given in ref. 10) defines β-sheets by their strand–strand interactions, rather than by single residue conformation, such as backbone (φ, ψ) angles. The protein coordinates are from ref. 11 (and updates as of August 1978). We identify two neighbouring antiparallel (or parallel) stretches of polypeptide chain (at least three residues each) as a pair of β_A (or β_P) strands (1) if pairs (i, j) of contacting residues have their side groups on the same side of the sheet (Fig. 1), and (2) if the C=O and N—H groups of the backbone are properly orientated[10]. Single strands are not counted. For regular stretches of

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Table 1 Amino acid residues in antiparallel and parallel β-strands

	Residue contact count			Frequency (%)				Conformational preference†							
	β_A	β_P	All β	β_A	β_P	All β	Global*	β_A	β_P	β_P/β_A	All β:	Here	Ref. 13	Ref. 14	Ref. 12
Gly	81	38	119	5.1	7.2	5.7	9.2	0.56	0.79	1.41		0.61	0.75	0.66	0.92
Pro	29	8	37	1.8	1.5	1.8	4.4	0.42	0.35	0.83		0.40	0.55	0.84	0.64
Asp	39	14	53	2.5	2.7	2.5	5.3	0.47	0.50	1.08		0.48	0.54	0.64	0.72
Glu	47	15	62	3.0	2.9	2.9	4.8	0.62	0.59	0.96		0.61	0.37	0.61	0.75
Ala	119	44	163	7.6	8.4	7.8	8.4	0.90	1.00	1.11		0.92	0.83	0.79	0.90
Asn	41	12	53	2.6	2.3	2.5	4.2	0.62	0.54	0.88		0.60	0.89	0.66	0.76
Gln	63	5	68	4.0	1.0	3.2	3.4	1.18	0.28	0.24		0.95	1.10	1.13	0.80
Ser	123	33	156	7.8	6.3	7.4	9.0	0.87	0.70	0.80		0.82	0.75	0.84	0.95
Thr	139	21	160	8.8	4.0	7.6	6.8	1.30	0.59	0.45		1.12	1.19	1.14	1.21
Lys	83	22	105	5.3	4.2	5.0	7.1	0.74	0.59	0.79		0.70	0.74	0.72	0.77
Arg	45	10	55	2.9	1.9	2.6	2.8	1.02	0.68	0.67		0.93	0.93	1.04	0.99
His	44	5	49	2.8	1.0	2.3	2.5	1.12	0.38	0.34		0.93	0.87	0.78	1.08
Val	188	108	296	11.9	20.5	14.1	7.8	1.53	2.63	1.72		1.81	1.70	1.97	1.49
Ile	112	63	175	7.1	12.0	8.3	4.6	1.54	2.60	1.69		1.81	1.60	1.95	1.45
Met	24	11	35	1.5	2.1	1.7	1.4	1.09	1.49	1.37		1.19	1.05	1.26	0.97
Cys	45	11	56	2.9	2.1	2.7	2.3	1.24	0.91	0.73		1.16	1.19	1.55	0.74
Leu	141	53	194	8.9	10.1	9.2	7.1	1.26	1.42	1.13		1.30	1.30	1.26	1.02
Phe	68	24	92	4.3	4.6	4.4	3.5	1.23	1.30	1.06		1.25	1.38	1.30	1.32
Tyr	98	21	119	6.2	4.0	5.7	3.7	1.68	1.08	0.64		1.53	1.47	1.49	1.25
Trp	47	8	55	3.0	1.5	2.6	1.7	1.75	0.89	0.51		1.54	1.37	0.90	1.14

The order of amino acids reflects their average structural similarity[17]. Amino acids which are observed to be more mutually exchangeable in evolution are nearer each other in this list.

* Numbers taken from ref. 11.

† The preference for $\beta_A(\beta_P)$ is a normalised frequency: the frequency in $\beta_A(\beta_P)$ divided by the global frequency. Note that the definitions of β-residues vary among different authors[7,13,15]. Furthermore, our preferences are for making β-sheet contacts, whereas those of the other authors are for occurrence in extended or β-strands.

β-strands (generally away from the edges of sheets) our identification of β-residues agrees with that of the crystallographers. As we define β-residues by their contact with neighbouring strands, we count residue contacts rather than residues in deriving the single residue frequencies. Thus, residues in strands at the edge of a sheet are counted once, residues in interior strands are counted twice, and residues making β_A contact on one side and β_P on the other are counted once for each type of contact.

The residue contact counts and residue contact frequencies are given in Table 1. β_A structures outweigh β_P by about three to one in the data base. The amino acid compositions of β_A and β_P are remarkably distinct. About one-third of all residue contacts in β_P are made by Val and Ile, the two non-polar side chains branched at C_β. Val (21%), Ile (12%), Leu (10%) and Ala (8%), with purely aliphatic side chains, together account for more than 50% of all contacts in β_P. In β_A six amino acids are needed to reach the 50% mark, including the polar side chains Thr and Ser, and the order of abundance (listed in decreasing order) is different: Val, Leu, Thr, Ser, Ala, Ile.

The 'conformational preference' parameters presented in Table 1 are defined as the ratios between the frequency of pair-contacts made by a residue in β-strands and the global frequency of occurrence of that residue anywhere. Values larger than 1.0 indicate preference of the particular residue for $\beta_A(\beta_P)$, values less than 1.0 indicate the opposite. Conformational preference parameters (derived from residue frequencies rather than from contact frequencies) have been used by many authors (for reviews see refs 4–6, 12) in the prediction of secondary structure (called 'potential', 'propensity', 'information measure'). The parameters given by Chou and Fasman[13], by Garnier *et al.*[14], and by Levitt[12] are included in Table 1 for comparison. There is overall agreement between the 'all β' (β_A and β_P together) preferences in the different studies. The differences, for example, for Pro, Glu or Trp, arise because the studies use not only different sets of proteins as their data base, but also different ways of identifying β-residues[7,13,15] and, in our case, different weights for interior and edge strands.

Comparison of conformational preferences between β_A (antiparallel) and β_P (parallel) shows: (1) the β_P structure is more selective: there are more extreme large and small values in β_P (standard deviation 0.66 for β_P but only 0.40 for β_A; high and low: 2.63 and 0.28 for β_P but only 1.75 and 0.42 for β_A), and, in β_P fewer residues are preferred (only 6, compared with 12 in β_A) and for these few the preference is stronger (except Tyr). In fact, the 20 amino acids can be divided into three groups (Table

Table 2 Conformational classification of β-sheet residues

		Conformational preference
Favourable in both β_A and β_P	Val Ile Met Leu Phe Tyr	>1.0 in β_A and β_P
Favourable in β_A but unfavourable in β_P	Gln Thr Arg His Cys Trp	>1.0 in β_A and <1.0 in β_P
Unfavourable in both β_A and β_P	Gly Pro Asp Glu Ala Asn Ser Lys	≤1.0 in β_A and β_P
Best sheet 'makers' in β_A	Trp > Tyr > Val > Ile	>1.5
Best sheet 'makers' in β_P	Val > Ile > Met	>1.5
Worst sheet 'breakers' in β_A	Pro < Asp < Gly < Glu < Asn	≤1/1.5
Worst sheet 'breakers' in β_P	Gln < Pro < His < Asp < Glu < Thr < Lys	≤1/1.5

2)—favourable in both β_P and β_A, favourable in β_A but not in β_P, and unfavourable in both β_A and β_P. (2) β_P 'abhors' polar and charged groups and favours hydrophobic side chains more strongly than β_A: only the purely hydrophobic residues Val, Ile, Leu, Met, Phe are significantly preferred (>1.2) in β_P, whereas in β_A also Thr, Cys, Tyr and Trp, all containing polar atoms, show values above 1.2. (3) Val and Ile are particularly favoured in β_P, with the second largest secondary structure enhancement values reported so far—2.6. Only the value for Pro at the second position in reverse turns[16] is larger—3.4. In β_A the preferences for Tyr and Trp (1.7) are exceptionally large. Note that both have a large non-polar ring in combination with a polar group. (4) The differences between β_A and β_P are particularly large for Gln, Thr, His and Trp (favourable in β_A and unfavourable in β_P); Gln is less favoured in β_P by about a factor of 4.

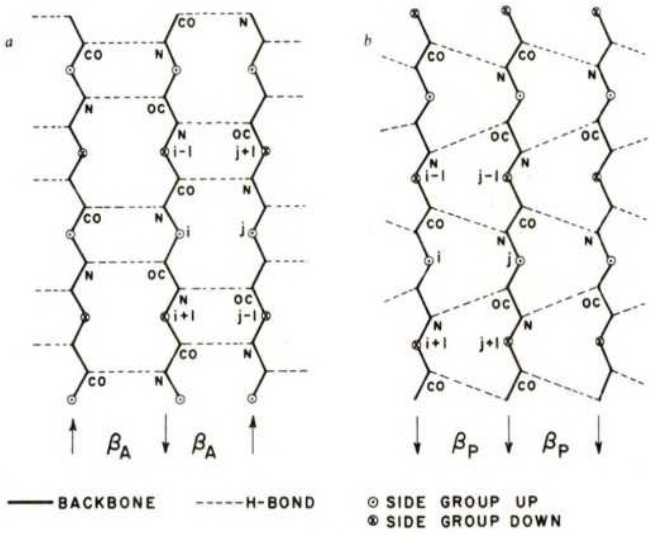


Fig. 1 A schematic drawing of antiparallel (*a*) and parallel (*b*) β-strands. The arrows indicate the N-terminal to C-terminal direction of the polypeptide backbone. Although the (ϕ, ψ) backbone angles are similar for β_A and β_P, the arrangement of the strands and the hydrogen-bonding patterns are fundamentally different. This difference is also reflected in different amino acid preferences.

The results presented here are potentially useful in two ways. On the one hand, they help to pose more specific questions, by focusing attention on those residues which have a special structural role. In this, residues which are both abundant and preferred are the most interesting. For example, one may ask: can Val and Ile serve as nucleation points for β_P-strands by restricting the backbone conformation with their side chains? Do they pack particularly well in the environment of the crossover connection between β_P-strands? Do they interact preferably with each other in forming clusters on the surface of β-sheets? The aim in investigating this type of question is to understand the physical basis of the statistical preferences.

On the other hand, the conformational preferences can be incorporated into structure prediction schemes, allowing distinct predictions for antiparallel and parallel β-stands. However, when this is done the limitations inherent in all statistical approaches to protein structure must be kept in mind: proteins are biologically evolved molecules with a particular structure tailored to a particular function. Only average properties of protein structures can be understood by statistical methods, but not the highly individual character of each protein.

Thus, the main conclusions regarding protein architecture are: (1) antiparallel and parallel β-strands are as distinct in their amino acid preferences as are α-helices and β-strands; (2) parallel β-strands impose much more severe constraints on their amino acid content than antiparallel β-strands or α-helices; (3) Val and Ile have an outstanding role in parallel strands. In a related paper[10], we analyse interstrand pair recognition of amino acid residues and its role in the tertiary structure assembly of strands into sheets.

As more protein structure data become available, further distinctions of secondary structure elements according to the type of tertiary contacts should be made. For example, one can distinguish different hydrogen-bonding positions in β-sheets, solvent-exposed and interior faces of sheets or helices, segments in tertiary contact with sheets compared with those in contact with helices. Such distinctions are likely to lead to more clearcut statistical preferences, and also serve as a starting point for predicting tertiary structure.

We thank John Moult, Barry Robson, Charlotte Schellmann and Joel Sussman for critical suggestions and Richard Feldman for supplying protein coordinates.

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Errata

The letter 'Local destabilisation of a DNA double helix by a T–T wobble pair', *Nature* **281,** 235, was printed with errors in the author line. This line should read:

A. G. Cornelis Haasnoot, Jeroen H. J. den Hartog, Jan F. M. de Rooij, Jacques H. van Boom & Cornelis Altona.

In the paper 'Isolation, cloning and sequence analysis of the cDNA for the α-subunit of the human chorionic gonadotropin', *Nature* **281,** 351, figures 1 and 2 should be transposed. The legends remain as published.

In the paper 'Unmasking of fetal determinants on adult bone marrow cells', *Nature* **281,** 484, the second author should be spelt B. M. Sheinberg.

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matters arising

Evidence for a higher natural uranium content in world rivers

THE letter by Mangini *et al.*[1] on aspects of the geochemical cycle of uranium requires comment. They attempt to show that phosphatic fertilisers do not contribute significantly to the uranium budget of rivers or groundwater, but the arguments and evidence they produce to support this claim are deficient.

They claim, from the observed phenomenon that rivers show a significant ^{234}U excess but that phosphate fertilisers do not, a contradiction to the assumption of a pollution effect. Yet the ^{234}U excess merely reflects the preferential leaching of this daughter product rather than its parent ^{238}U. Since this fractionation was noted in 1953[2] it has been developed as a uranium exploration tool[3] and a dynamic equilibrium model[4] has been developed showing its variation about source rocks which themselves have an average AU ~ 1 (AU, ratio of ^{234}U to ^{238}U).

A similar claim is made that the close relationship found by my colleagues between uranium content and bicarbonate (HCO_3^-) content[5] for rivers and groundwaters indicates a natural origin for the uranium. Again, this relationship merely shows the common mode of migration of uranium in solution as a bicarbonate complex and gives no evidence for the source of the uranium.

The lack of a clearcut relationship between the uranium and phosphate contents of the water is further taken as evidence for a negligible contribution of uranium from phosphatic fertilisers. Again this is a function of the relative solubilities of the uranium and phosphate components and itself provides no evidence for the source of the uranium. In the Devonian sediments of northern Scotland there are naturally occurring bedded concentrations of uranium in phosphatic rocks[6,7] and related uranium anomalies are present in the surface water, but these exhibit no corresponding anomalies in phosphate content. There is thus selective leaching of uranium from these bedded phosphatic sediments.

Additionally, both in the Caithness and Orkney areas a limited number of spuriously high values were detected (>10 to 100 $\mu g\ l^{-1}$ U) and these were related to transient enrichments from fertilised areas. Samples of phosphate fertilisers used in the area contain up to 100 μg U per g. This was taken as supportive evidence for the regional scale contamination noted by Spalding and Sackett[8], but rejected by Mangini *et al.*[1].

Rock phosphate (apatite/collophane) is able to fix uranium in both oxidising and reducing conditions. Uranium can be present both in the U^{4+} form, replacing Ca^{2+} in the apatite structure, and as U^{6+} where it is adsorbed on the surface. It is the adsorbed uranium which is readily removed by surface waters. Experimentally Spalding and Sackett[8] showed the ease with which uranium may be leached by water from superphosphate fertiliser. They also demonstrated that after application of fertiliser and 0.5 inches of rainfall equivalent, the uranium level in the run-off was 28 times greater than in a similar blank experiment.

Mangini *et al.*[1] present groundwater evidence from a highly fertilised area which does not show any enhancement of uranium in the subsurface waters and take this to indicate that "more than 95% (2σ) of uranium supplied with the fertilisers is retained in the uppermost soil layers". They also note that "less than 0.01% of the phosphate goes into groundwater" [I presume ND in their Table 1 means 'not detectable' and not 'not determined' as stated] and state "assuming a similar transport mechanism for uranium the contamination would make less than 0.004 $\mu g\ l^{-1}$". As indicated in the preceding paragraphs this assumption must be rejected as uranium is leached separately from the phosphate.

The explanation for the low uranium values in the groundwater, although the added fertiliser contains 50 p.p.m. U, may simply be that the uranium is adsorbed and complexed from the groundwater in the soil profile but could still be being leached by the surface run-off. No figures for uranium in the surface run-off are presented and, as the area has been fertilised for several decades, the original background may not be determinable. A transient increase following application of fertiliser should be sought.

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MANGINI REPLIES—Our conclusion that in the Handschusheimer Feld area the influence of uranium from phosphate fertilisers is small was derived both from the low uranium contents of groundwater and river water, more importantly, from their high AU (~1.6).

We appreciate the opportunity to clarify a point to those not familiar with ^{234}U and activity ratios (AU). ^{234}U is a member of the ^{238}U decay series, with a half life of 244,000 yr. Disequilibrium between ^{234}U and ^{238}U ($AU \neq 1$) is a common phenomenon in nature. Rocks and clays from which uranium is being extracted have $AU \leq 1$, rivers and groundwaters $AU > 1$ (average 1.20) and recent uranium deposits (younger than 10^6 yr) $AU \geq 1$. In uranium-enriched phosphate fertilisers we find, as expected, AU ~ 1.

Preferential selective leaching of ^{234}U from rocks and clays is attributed to the α-recoil on the ^{234}Th nucleus, following the ^{238}U α-decay. Recoil energies of approximately 0.1 MeV 'shoot' ^{234}Th (the short-lived grandmother of ^{234}U) from a 550-Å thick surface layer of the mineral grains into solution[1]. Whenever traces of uranium are leached, the solution will have $AU \geq 1$.

The situation is different in the upper soil layers, where uranium is being supplied by fertilisation at AU ~ 1.

In fact a competitive fertiliser should nearly completely dissolve in a period of months. The consequence is that nearly all uranium (^{234}U and ^{238}U) undergoes dissolution (as confirmed by Spalding and Sackett's experiments) and, we believe, will be adsorbed and complexed in the uppermost soil layers—^{234}U in the same chemical form as ^{238}U. If, either by groundwater or surface run-off from this soil, any leaching of uranium occurs, dissolved uranium should have the same AU (~1) as the soil, as there is no chemical difference between complexed ^{234}U and ^{238}U. Even after, say 1,000 yr, less than 3‰ of ^{234}U will be the more easily leachable 'recoil ^{234}U'.

Tritium measurements on precipitation and stream water in the Neckar system indicate that, for heavy precipitation, surface run-off contributes less than 5% to the river discharge. We presume that most of this run-off contribution originates in the inclined areas of the river spring system where horticulture should be negligible[2].

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reviews

Born's intimate reminiscences

P.S. Farago

My Life: Recollections of a Nobel Laureate. By Max Born. Pp.308. (Taylor and Francis: London; Scribners: New York, 1978.) £8.50; $17.50.

THE basic facts about Max Born's career can readily be found; there is an entry under his name in every respectable encyclopaedia. One aspect or another of his pioneering work is well known to any competent physicist; many received their first introduction to atomic and molecular phenomena from his *Atomic Physics.* Born himself wrote several retrospective essays, some of which are collected in the volume *My Life and My Views;* and an excellent study of his life and works appeared in 1971 in the Biographical Memoirs of the Fellows of The Royal Society. Yet there has been relatively little information about the forces which shaped his character and outlook, the motives which turned him from an average school-boy into a physicist of the greatest distinction, or his relations with the contemporary academic community and society at large. These and many similar questions are answered in this volume.

Max Born had just turned 50 and was at the height of his career when Hitler rose to power, and a few months later he was deprived of his position. He writes: "All I had built up in Göttingen, during twelve years' hard work, was shattered. It seemed to me like the end of the world". And in a way it was. He left Germany, and during his 20 years of exile he found no true spiritual home. After three years of limited security in a Cambridge lectureship he was appointed to the Tait Chair of Natural Philosophy at the University of Edinburgh in 1936. He stayed there until his retirement at the age of 70. The major part of the recollections, under the title "Good Old Days", was written here during the War. It takes the story up to 1925, including the birth of Quantum Mechanics. The second part, entitled "Tempestuous Years", is a more sketchy account of the rest of his active life, written in retirement in Germany when he was about 79. The Preface by his son tells us that these memoirs were written primarily for his family. Indeed the reader feels as if he were admitted to this intimate circle to witness Born re-living his eventful life. The narrative is loosely spun of several strands and presented in a discursive style.

Firstly, the story of his family. On his mother's side, rich and influential industrialists enjoying a life of luxury, highly educated amateurs and patrons of the arts (mainly music and literature), friends of and hosts to eminent artists of the time. "My father's ancestry", he writes, "was humbler in regard to all things of property and consequence in the world, but I believe that the essence of my nature is due to the Born clan". As the narrative unfolds there emerges a view of the social and political scene in Prussia about the turn of the century, and in Germany as a whole during the following decades.



Max Born in the 1920s

Against this back-drop one can see the working of the factors which shaped his remarkable character. A fixed reference frame was defined for him by the traditions of his Jewish ancestors, emancipated generations earlier. "They believed they differed from their neighbours only in respect of religion, for which they had hardly any use, and they were Germans by culture and custom". This deep-seated sense of identity, an "inextinguishable homesickness for the German language and landscape", played an important part in deciding him to return to Germany after his retirement.

Born's concept of right and wrong began to be formed at a tender age and crystallised early, under his father's guidance. He recalls touching examples of his father's methods of teaching "practical ethics". The moral code thus assimilated did not conflict in any way with the enjoyment of beauty in nature and in art, and with taking a fair share of all the pleasures of a full life.

Intertwined with the family saga there appears a vivid picture of the contemporary educational system, and of academic life with its social and professional facets, depicted first from the point of view of a student, and then as seen by a scientist of rapidly growing stature. Fascinating portrait sketches bring to life many unique individuals, his teachers, fellow students, eminent colleagues of several generations, and highly gifted pupils. Some are portrayed with affection and respect, some observed with critical eyes and some drawn in the style of mocking cartoons.

As the narrative progresses he overcomes his apparent reluctance to 'talk shop'. Bit by bit there emerges a complete, though non-technical, survey of his own work and his own assesment of its significance. Reading these passages one can catch a glimpse of the way his creative intellect worked and of the emotional response to recognition given or denied to him at various stages of his career. Some of his attainments he thought were overrated, others were not appreciated adequately. Above all, the lack of recognition for his contribution to the foundation of the principles of Quantum Mechanics left a very deep and long-lasting scar, healed only by the Nobel prize 28 years later, during his retirement.

Max Born and his contributions to our knowledge of the physical world became enduring features of the history of science. This volume of his reminiscences reveals a whole world unknown to the present generation, and through it one can piece together the complex personality of the narrator. His reflections on a philosophical seminar he attended as a student in Göttingen could stand as his testament: "I can quite well grasp Kant's idea that there are principles or categories of thinking which are the conditions of actual knowledge and which you can discover by investigating the structure of this knowledge. That is what we theoretical physicists are really doing — with the sole difference that we do not claim that our latest analysis is a final and irremoveable law, but just another step to a remote truth. To look for ultimate evidence by introspection, contemplation and verbal analysis, is irreconcilable with science, and seems to me in practical life the source of all ideological struggle. For anyone who believes he has obtained such an internal evidence easily becomes a fanatic, a mystical believer unapproachable by reason and argument". □

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Parasite physiology

Biochemistry and Physiology of Endoparasites. By Theodor von Brand. Pp. 447. (Elsevier/North Holland/Biomedical: Amsterdam; New York and Amsterdam, 1979.) $87.75; Dfl.180.

THEODOR VON BRAND died in 1978 and parasitology lost the man who had pioneered the study of the physiology and biochemistry of parasites and whose name had become synonymous with the subject for almost forty years. von Brand was probably the best person to be able to overview the whole of this area of study and fortunately he managed to complete the text of this book before his death. This remarkable synthesis of a massive amount of information will remain as a tribute to the man himself and will join his previous works, *Chemical Physiology of Endoparasitic Animals* and *Biochemistry of Parasites,* two of the most influential books ever written on parasitology.

Biochemistry and Physiology of Endoparasites is an updated version of *Parasitenphysiologie* published in German in 1972. There are ten chapters each with between 170 and 550 references mainly from the 1960s and 1970s. The chapters include the obvious ones on nutrition, respiration composition, synthetic processes, degradation processes, and sensory, nervous, muscular and locomotory physiology, and less obvious ones on parasitic habitats, hormones, pathophysiology and the physiological basis of chemotherapy. There is therefore something of interest for everyone. Von Brand wrote beautifully and was also a master of synthesis: the essentials of whole papers are expertly digested and summarised in a few words so carefully chosen that anyone can understand them. This is a book for casual reading and for reference but it is as the former that it will be most useful. The committed parasite physiologist will have access to specialist reviews and literature retrieval facilities but even he should be able to use this book to put his work into some sort of general context.

A book of this kind cannot, of course, please everybody and it is stronger on helminthology than protozoology, physiology than biochemistry, and physiology proper than the rather newer fields of pathology, immunology and chemotherapy. This uneven coverage is inevitable and probably reflects real differences in our depth of knowledge of various aspects of parasitology.

It is customary today to criticise the price of books and in this case the publishers have done a considerable disservice to parasitology, which by its very nature is a subject of particular importance in the poorer parts of the world. The price will limit the purchase of this book to specialists who, for reasons given above, will learn least from it. This is a pity because this book summarises the state of the art; and as this subject is expanding so rapidly the preparation of a revised edition will be an impossible task. It is to be hoped that the publishers will find it possible to produce a cheap version for parasitologists in the tropics and students.

Had this been a cheap book the mistakes in it could have been forgiven but there are too many of them and they do mar the book and reduce its value as a work of reference. For example, there are inconsistencies in the spelling of names (Bernstein and Baernstein, Buelbring and Bülbring); the sources of figures and diagrams are given but do not appear among the references (Smyth (1969) on page 196 and Ryley (1967) pages 184-185); the names of parasites are not given in the captions to some of the figures (Figs. 41 and 49); and worst of all, and proof that these mistakes are not those of von Brand himself, his name appears under the Bs in the references to chapters 1 and 2 and the Vs elsewhere.

These shortcomings, however, are trivial compared with the importance of this book as a whole. No-one else will ever write a book of this kind and no parasitologist should fail to read it.

F.E.G. Cox

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Reichenbach's writings

Selected Writings, 1909-1953. Two volumes. By Hans Reichenbach. Principal translations by Elizabeth Hughes Schneewind. Edited by M. Reichenbach and R. S. Cohen. Pp. 500+435. (D. Reidel: Dordrecht, The Netherlands, Boston, and London, 1979.) Vol. 1: hardback Dfl. 145, $64.50; paperback Dfl.60, $27; Vol. 2: Dfl. 130, $59 and Dfl.60, $27.

HAD Hans Reichenbach (1891-1953) not died unexpectedly at the age of 62, a volume on him in the Library of Living Philosophers would have certainly gone beyond the stage of preliminary planning. According to the structure of volumes in that Library, an autobiography of Reichenbach would have been followed by a series of essays by other philosophers on the various aspects of his thought and a reply by him in addition to a list of his publications. This two-volume set contains such a list and starts with an autobiographical sketch followed by over thirty reminiscences by members of his family, friends, colleagues, and students which cover his childhood, university years, his first teaching post in Stuttgart's Technische Hochschule, his tenure at the University of Berlin, his stay from 1933 until 1938 at the University of Istanbul, and finally his years as a widely acclaimed professor of philosophy at the University of California in Los Angeles and a chief spokesman of logical positivism in the United States.

These reminiscences are full of illuminating gems about Reichenbach's life, work, thought, and personality which only the eyes of admirers could discern and retain for posterity. Anyone nurturing the notion that the Vienna Circle, of which Reichenbach was a prime mover as the founder of its organ, *Erkenntnis*, was given entirely to abstractions, can now learn from a most reliable source that Reichenbach considered himself a crusader. On hearing himself described as one whose sole interest was logic and epistemology, he reacted angrily: "But no! That is not true! The whole movement of scientific philosophy is a crusade . . . what I am doing aims as directly at social consequences as the programs of those who call themselves social reformers". The hitherto untranslated or no longer in-print publications of Reichenbach, which make up the bulk of these two volumes, have as their first group eight essays on social problems, written before and during World War I, with a strongly Socialist though not Marxist accent.

Equally instructive is Reichenbach's characterisation of the indispensability of philosophers: they (he means above all himself — witness his *The Rise of Scientific Philosophy* in which the modern part is largely limited to his own philosophy) are alone capable of articulating what the scientists, more often than not, brilliantly do but clumsily say. Reichenbach was a master of articulating not only the most abstract scientific notions and procedures (he had his own programme at the State Radio in Berlin), but also of handling down-to-earth matters. An engineer by training (he did not want to become an engineer "who can do nothing but calculate") he was competent in household repairs. He could deliver impromptu speeches on most obtruse topics, had touching sensitivity for others' needs, did pioneering work in interdisciplinary teaching, but, as stated in one of the reminiscences, was possessed "like most philosophers . . . of a strong sense of vanity".

The pride and centre of Reichenbach's philosophy was the probability theory of existence. He came to it partly through his early fascination with the kinetic theory of gases, partly under the impact of quantum theory, and partly because he took Einstein's relativity as a proof that questions about reality as such were meaningless. It was through Einstein's

theories of space and time that Reichenbach perceived the errors of Kantian metaphysics.

The items offered in Part II and Part III under the respective titles "Popular Scientific Articles" (21 items published between 1922 and 1932) and "General Scientific Articles" (11 items of which 10 were published between 1925 and 1933) give much insight into both Reichenbach's effort to develop a "scientific" philosophy, whose sole criterion of truth was physics, and into his keen interest in the latest developments of science.

Reichenbach is always his own voice even when he speaks of others, a desirable stance when taken by a great mind, but also rich in unintended instructiveness. The best example of this is provided in Reichenbach's writings on Einstein, his hero, whom he knew at close range, as he had attended with four others Einstein's first seminar in Berlin. There is no trace in these two volumes — judiciously selected, brilliantly translated, carefully edited and annotated — that Reichenbach had ever taken heed of the increasingly more emphatic endorsements by Einstein of a metaphysically realist epistemology and of his summary dismissals of positivism, including its "logical" brand.

The historian of science will not be surprised on finding that Reichenbach never makes as much as a modest effort to take a critical look at inherited clichés relating to scientific history (his reading of Kant's early scientific writings is very superficial indeed) and to live up to his professed premise to be respectful only of carefully established facts. Though largely uninterested in history, he did not, unlike some others in the Vienna Circle, forge facts (to recall a famous 'confession' of Feigl) to suit theories.

Two articles on ethics and free will, which as Part IV complete Volume I, are for all their brilliance in argumentation a perfect case of myopia concerning the circularity in which Reichenbach's classification of moral judgments as volitional decisions is caught. Is not Reichenbach's *own* decisional definition of cognitive meaning the very basis of that classification?

Volume II, which contains Part V ("Philosophy of Physics") and Part VI ("Probability and Induction"), is harder reading, but the gleanings are rich both for admirers and critics. The question of "now" as handled by Reichenbach in an essay of 1925 is a case in point. The existential, irreducible aspect of "now" never comes within his ken. The sole alternatives for him are choices between mathematical techniques, which he handles superbly, appropriate for deterministic and probability sequences, respectively. It was, of course, much later that Einstein reminded Carnap about "now" as something which slips through the nets of physics, but not so late that Reichenbach, a close friend of Carnap, could not have heard of it.

The most notable item in Volume II is a translation, under the title "The Aims and Methods of Physical Knowledge," of an essay originally published in 1929 in the section of the famed *Handbuch der Physik* dealing with the general foundations of physics. Reichenbach was obviously well known and highly regarded in the Germany of physicists, then the home of many major advances in theoretical physics. The very title of that essay cannot help remind one of a classic book of Duhem which was well known in German translation in the Vienna Circle. In the more than a hundred footnotes and bibliographical references of that essay, Duhem's name occurs not once, nor in the two volumes for that matter, a telltale sign of the narrowness which logical positivism can generate.

The last item in Volume II is a letter which Reichenbach wrote on March 28, 1949, to Bertrand Russell. Reichenbach hoped that a discussion of an hour or two would settle the dispute between them and then, as Reichenbach put it, "you would see that your abandonment of empiricism is unnecessary and that you need not resort to an extra-logical principle not based on experience". A vain hope, indeed. Russell had since long achieved a better grasp of the limitations of logic and method than the best which Reichenbach could ever muster.

Stanley L. Jaki

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Analytical flame spectroscopy

Fundamentals of Analytical Flame Spectroscopy. By C. Th. J. Alkemade and R. Herrmann. Pp. 442. (Adam Hilger: Bristol, UK, 1979.) £25.

THIS book makes an extremely good and successful attempt to treat the three branches of analytical flame spectroscopy — atomic emission, absorption and fluorescence — in a unified way. It provides the novice and afficionado alike with a sure guide to the understanding of the phenomena involved in atom production within chemical flames and their subsequent excitation for emission, absorption or fluorescence.

There are many books that treat analytical flame spectroscopy, but this one is probably unique in that it deals almost exclusively with the fundamental aspects of flame spectroscopy as they relate to practical analysis. Early on, the authors make the point that, although it is relatively easy to establish an analytical method based on atomic spectroscopy for the determination of a given trace element under defined circumstances, it is not so easy to extrapolate it to other conditions or to explain interferences and limitations. It is also difficult usually to establish successful adaptations without a knowledge of the fundamental processes that are involved. To this end the text incorporates the most relevant results of fundamental studies in atomic spectroscopy, the physics of collisions, chemical kinetics, combustion research, and so on. There are chapters on flame structure, pneumatic nebulisation, desolvation and volatilisation of analyte species, dissociation and ionisation, background emissionand absorption, shapes of analytical growth curves, and the classification of the interferences of concomitant substances. Throughout this treatment the three branches are dealt with synoptically or in juxtaposition so that their relative merits or limitations stand out clearly. There are also numerous references to the corresponding phenomena in electrothermal atomisation, thus enabling the reader to make his own assessment of the alternative (non-flame) atomisation technique.

The level of treatment assumes a basic knowledge of physics and chemistry that is possessed by most graduate students or experienced analysts who use atomic spectroscopy; it should not cause many problems to the average reader in this respect. It is also a matter of considerable satisfaction to find the authors using the terminology and nomenclature recommended by the Spectrochemistry Commission of the Analytical Division of the International Union of Pure and Applied Chemistry. These terms are defined in a glossary which reproduces the IUPAC report and, additionally, cross-references the earlier terms which abound in the literature. This section comprises 223 pages and is followed by a useful list of flame spectrograms showing the emission spectra of flames, hollow cathode lamps and atoms in flames. An index of flame spectra (lines and bands) is provided for the range 177.495-1252.2 nm to facilitate the identification of unknown peaks; and, finally, there is a comprehensive list of flame spectra arranged in alphabetical order of the elemental symbol. Over 900 references are cited and there is a comprehensive subject-author index.

There is no doubt that this is an extremely valuable book for background reading and general information for all who use analytical atomic spectroscopy in flames.

T.S. West

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Comprehensive survey of the pulmonates

Pulmonates. Volumes 2A and 2B. Edited by V. Fretter and J. Peake. Pp. 540 and 150. (Academic: London, and New York, 1979.) £24.50, $50,.75 and £12, $25.20.

ANIMALS with high potential for colonising the land came into existence with the first appearance of the Gastropoda, of which the pulmonates form a subclass. The compact body was protected by an impervious shell with a restricted opening into which the broad foot was withdrawn; and the enclosed mantle cavity easily converted into a repiratory sac. Emancipation of the reproductive from the renal system made internal fertilisation possible and removed the last barrier to landward passage.

The subclass Pulmonata is classified into two orders. The modern Stylommatophora possess unique ingenuity in resisting the prime danger of dessication; and it is a rare terrain that is devoid of these animals capable of remaining dormant for the greater part of the year, even for years on end. In contrast the Basommatophora have returned to aquatic life — largely in freshwater, but in certain seas they are amongst the commonest intertidal limpets.

Dependence by the majority of snails and slugs on plant life has made them major competiors with man for food; and their association with other animals, notably in damp places, has made them admirable intermediate hosts for parasites. They are thus animals of high economic significance and for that reason also worthy of careful study.

These volumes represent the completion of a comprehensive survey begun in 1975 with the publication of a volume dealing with functional anatomy and physiology. In volume 2A treatment covers *systematics, evolution and ecology* presented in 10 chapters by highly competent authors.

With unique wealth of experience, B. Hubendick reviews systematics and comparative morphology in the Basommatopora revealing the range of structural variety from high spired snails to flattened limpets. This is followed by consideration from the equally authoritative pen of A. Solem of the data on which classification of the 20,500 odd species of land molluscs, almost entirely Stylommatophora, is based. In both chapters, unfortunately, references are largely restricted to those before 1972.

G.M. Davis, concerned with precise demarcation of species where transmission of human parasites is involved, reveals the importance of the new methods of chromatography, electrophoresis and immunology. This leads on to an account by C.M. Patterson and J.B. Burch of the results of work on the chromosomes of pulmonate molluscs. Chromosomes vary from 5 to over 70 pairs although variation is ussually conservative within the various taxa. B. Clarke and co-workers from Nottingham deal with genetic variation, showing how pumonates are particularly favourable material for demonstrating the action of natural selection on genetically determined visible characters.

Convergence is widespread throughout the Mollusca, the diversely originating slugs being notable examples. Liable to become major pests, control, as P.J. Hunter stresses, often depends on knowledge of their ecology. This leads on to the chapter by D.S. Brown on pulmonates as intermediate hosts for digenetic trematodes. No-one is better qualified than W.D. Russell-Hunter to discuss the ecology of freshwater pulmonates, and so we proceed by way of an account of the evolution of gastropods in ancient lakes by K.J. Boss to the final chapter on the distribution and ecology of the Stylommatophora by J. Peake, co-editor with Vera Fretter of these volumes.

Volume 2B entitled *Economic Malocology,* by A.R. Mead represents a continuation of its author's wonderful Giant African Snail (*Achatina fulica)* published in 1961. This supreme malacological saga tells of the spread by a surprising variety of agencies across Asia and then over the Pacific islands of this relatively immense high spired snail. Its supreme attempts to enter the United States, first by way of California and more recently by way of Florida, were only with difficulty repelled. This volume brings the story up to date and adds to the debt all malacologists owe to its author.

Enough one hopes has been written to reveal the range and high competence of these volumes and their importance to zoologists working on both pure and applied aspects of their subject.

C.M. Yonge

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Peptide synthesis

The Peptides. Edited by Erhard Gross and J. Meienhofer. Pp.435. (Academic: New York and London, 1979.) $39.50.

THE publication of *The Peptides* by Schröder and Lübke in 1965 was a landmark in the literature of peptide synthesis. The intervening period has seen the continued rapid growth of the subject, both in the development of new methodologies (including solid-phase synthesis) and in biological importance, yet no major survey in the English language has appeared. This entirely new *The Peptides* edited by Gross and Meienhofer is thus particularly welcome. The overall scope of the multi-volume and multi-authored work has been widened as indicated by its subtitle, *Analysis, Synthesis, Biology*; this first volume deals with major methods of peptide bond formation.

The opening chapter (by Gross and Meienhofer) presents a broad survey of the properties of the peptide bond. The coverage is wide, embracing structure, cleavage and synthesis, and is necessarily superficial in some instances. A case in point is the section on conformational analysis in solution where the single example quoted (oxytocin) is atypical and gives a misleading impression of the field as a whole. It would now be generally agreed that in the absence of special constraints, short linear peptides have rather random conformations in free aqueous solution. Conformational analysis under these circumstances is less useful.

The following chapter (by J.H. Jones) surveys all the main methods for peptide bond formation. Much of the information is covered in greater detail in subsequent chapters. The author has perhaps wisely avoided overmuch comment on the relative merits and applicability of the various methods, though his personal experiences have been distilled most valuably into a section on the choice of individual activated esters. This is a useful introductory chapter, especially for the newcomer to peptide synthesis.

The following four chapters provide detailed coverage of the major methods of synthesis. An authoritative account by Bodanszky surveys a wide range of activated ester derivatives concluding with a most useful series of fully referenced tables. The azide and mixed carbonic anhydride methods are surveyed by Meienhofer. Inclusion of significant experimental detail in these chapters gives an excellent 'feel' for the various techniques. The carbodiimide method is reviewed by Rich and Singh. The volume is completed by a comprehensive account of the racemisation problem by D.S. Kemp.

It is inevitable that *The Peptides* will be essential reading for all engaged in or contemplating peptide synthesis. It will supplement (but not replace) the monumental reference work edited by Wünsch in Volume 15 of *Houben-Weyl's Methoden der Organischen Chemie* (1974). Further volumes of Gross and Meienhofer's compilation are awaited with great interest.

R. C. Sheppard

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obituary

Sin-itiro Tomonaga, 1906-1979

SIN-ITIRO TOMONAGA, who shared the Nobel Prize for Physics in 1965, died on 8 July 1979 at the age of 73. His memory will be cherished for many years to come because of his witty personality and his sparkling ideas which contributed to the development of quantum electrodynamics in the post-war period.

Tomonaga was born in 1906 in Tokyo as a son of an eminent philosopher, and moved to Kyoto in 1913 with his family. In 1923 he entered the Third High School, and in 1926 he was admitted in Kyoto Imperial University where he specialized in physics. Through these years he became acquainted with Hideki Yukawa, another Nobel laureate, and they studied together. They remained lifelong friends.

It was in 1931, two years after Tomonaga graduated from the university, that he met Yoshio Nishina. Nishina, fresh from Bohr's Institute in Copenhagen, visited Kyoto to give a series of lectures on quantum mechanics. He brought back not only the celebrated Klein-Nishina formula but also the stimulating Copenhagen spirit to Japan and inspired in the young audience a fascination for the new frontier. This gave Tomonaga an excellent opportunity to enjoy Nishina's generous personality, so that the following year he decided to accept an invitation from Nishina to work at the Institute of Physical and Chemical Research in Tokyo as his research associate.

Nishina directed Tomonaga's interest to quantum electrodynamics, and they started to work together on this subject. This was the beginning of Tomonaga's life work, for which he was later awarded the Nobel Prize. Those stimulating days in the free atmosphere of the institute shaped a fertile brain that came into blossom a decade later. Tomonaga often recollected that the joint colloquia at the institute were a constant inspiration to him.

In 1937, he went to Leipzig to join the theoretical group led by Professor Werner Heisenberg and stayed there for two years. During his sojourn there he published a paper on thermal and mechanical properties of nuclear matter. In 1939, after his return home from Europe he was awarded the degree of Doctor of Science from Tokyo Imperial University upon presentation of his dissertation based on the outcome of his stay in Leipzig. In 1940, he married Ryoko Sekiguchi, a daughter of the then director of the Tokyo Astronomical Observatory. In 1941, he joined the



faculty of Tokyo Bunrika University which was later absorbed into the Tokyo University of Education in 1949.

At that time (1941) he concentrated his thoughts on a non-perturbative approximation method in meson theory which developed afterwards into the strong coupling theory, as well as on the generalization of Dirac's many-time formalism to field theory. In the same year World War II broke out, and everybody had to get through a very difficult time. However, Tomonaga's spirits were high in those days, and he was widening his repertory of specialities ranging from field theory to electronics. He completed a relativistically invariant formulation of the quantum theory of wave fields, the so-called super-multiple-time formulation, as the ultimate form of Dirac's many-time formalism. He had to wait for the war to end, however, before his theory could bear fruit: the renormalization programme. During the war he developed a theory of microwave systems as an application of the S-matrix formalism. He also clarified the oscillation mechanism of magnetrons on the basis of an intuitive model and secular perturbation. For these contributions he was awarded the Japan Academy Prize in 1948 together with his collaborator Masao Kotani.

When the war was over in 1945, Tokyo had been reduced to ashes, and everybody had to go through all kinds of hardships. Research on nuclear physics was banned in Japan, and the cyclotron built by Nishina was dumped in the Bay of Tokyo. Yet Tomonaga reached the loftiest pinnacle of his scientific activities in this post-war period. His deep-rooted idea on the renormalization programme was ripe, and he immediately started to work towards completion of his theory with his young associates. They gathered to hold seminars in the midst of ruins. At that time Japanese physicists had almost no access to scientific journals from abroad, but Tomonaga learnt of the discovery of the Lamb shift and its interpretation by Bethe through the popular science column of an American weekly magazine. This hint was sufficient to convince him of the correctness of his idea and soon he established his version of quantum electrodynamics. Another fruit was the formation of the Tomonaga school through his collaboration with young physicists in this period.

In 1949, Tomonaga was invited to the Institute for Advanced Study in Princeton and stayed there for a year. There, he broke new ground in the many-body problem.

In 1951, Nishina died, and Tomonaga had to assume many of his administrative responsibilities. After reaching the highest scientific achievement, Tomonaga then paid special attention, in Nishina's place, to the improvement of the working conditions of young scientists. He dedicated his best efforts to the cause of starting research laboratories and facilities such as the Norikura Cosmic Ray Observatory and the rebuilding of a cyclotron at the Institute of Physical and Chemical Research. He also took the leadership in establishing the Institute for Nuclear Study and later the National Laboratory for High Energy Physics.

In 1956, he was elected President of the Tokyo University of Education and remained in this position until 1962. Then he served as President of the Science Council of Japan from 1963 to 1969. Since 1964 he was President of the Nishina Memorial Foundation established to encourage young atomic scientists. This was the position that he held until his death. In 1965, Tomonaga shared the Nobel Prize for Physics with Julian Schwinger and Richard P. Feynman. His scientific achievement was honoured also with the Order of Culture, Japan, in 1952 and the Lomonosov Medal, USSR, in 1964. He

was a member of a number of academies and learned societies both domestic and foreign.

Almost a year ago I visited him in a hospital and we talked about a book he was preparing, but then I had not the slightest idea that I was never to see him again.

Kazuhiko Nishijima

Gilbert Stead

PROFESSOR STEAD, who died on 5 July 1979, aged 91, was one of a small group of physicists who helped to establish radiology as an independent medical discipline.

Medical interest in X-rays was sparked off by a lecture given by Silvanus Thompson to the Clinical Society of London in March, 1896, and during the next 25 years, largely through the enthusiasm of younger doctors with no special training, the use of X-rays for diagnostic purposes spread rapidly. These pioneers often built and maintained their own apparatus!

It was not until the Cambridge Diploma in Medical Radiology and Electrology (D.M.R.E.) was instituted in 1919 that such training became available. A course in radiological physics was begun by J.A. Crowther at the Cavendish Laboratory in 1920, with Rutherford's active support; S. Russ and L.H. Clark provided similar courses in London. In 1924, Stead took over the Cambridge teaching as University Lecturer in Physics as Applied to Medical Radiology and he continued it until 1938. From 1927 until 1942, when this Diploma was discontinued, he was also Secretary to the Diploma Committee.

Stead's connection with Guy's Hospitasl began in 1923 when he was appointed Reader in Physics in the Medical School, a part-time post. The facilities available there only allowed for under-graduate teaching, but in 1938 he was elected by the University of London to a Chair of Physics and was given proper laboratory accommodation. Unfortunately, the outbreak of war in 1939 caused evacuation to Tunbridge Wells and it was not until 1945 that he could begin to build up his department as a centre for applying physics in medicine.

He set up courses for the newer radiological diplomas which had been instituted by the University of London and by the Royal Colleges of Physicians and of Surgeons; and he acted as examiner for them at various times until he retired in 1953, having contributed greatly to setting the standards of teaching and examination which made these diplomas universally accepted. He was President of the British Institute of Radiology (the original Röntgen Society, founded by a group of medical men in 1897) in 1947/48 and delivered the Silvanus Thompson Memorial Lecture there in 1959. He was Honorary Consulting Physicist to Guy's Hospital and a Governor of the Medical School.

During his 30 years at Guy's Stead established a great reputation as a teacher. Some 3,000 undergraduates (who had to "do" physics in their first year, not always willingly!) attended his courses. Gentle in manner, patient and understanding their difficulties, he succeeded not only in overcoming their foibles but also in winning their affection. A text-book, *Elementary Physics*, which he wrote with such students in mind, is now (1979) in its eleventh edition; it was first published in 1923!

Stead outlived most of his contemporaries, but many younger people, not only in this country, remember his retiring, courteous personality. He was once described as "shrewd in discussion, wise in counsel and temperate in judgment." Those who had the privilege of working closely with him knew him also as a loyal friend.

C.B. Allsopp

Carl L. Hubbs

PROFESSOR CARL LEAVITT HUBBS, one of the world's most distinguished ichthyologists, doyen of that tribe in America, a true naturalist and a great personality, died on 7 July 1979 at the age of 84.

Born in Williams, Arizona, shortly after his mother was rescued by a train crew who had found her lost in the desert while driving a wagon, Carl Hubbs ended his distinguished career as Professor Emeritus of the Scripps Institute of Oceanography (University of California) where he had worked since 1944.

The early arousal of his interest in natural history he attributed to his maternal grandmother, one of the first woman physicians; it was further expanded by his father, a self-taught mineralogist, and was fully developed at Stanford University under the rigorous tutelage of that master ichthyologist David Starr Jordan. Hubbs obtained his bachelor's (1916) and master's (1917) degrees at Stanford, and his doctorate (1927) at the University of Michigan. After three years as a curator in the Field Museum, Chicago, he returned, for 24 years, to the University of Michigan. There, apart from his lecturing duties he was curator of vertebrates in the University Museum, and was responsible for building up that museum's collection of fishes, now one of the finest in the world.

Hubb's command of ichthyology was awesome, his research interests extensive but penetrating; their results, published in over 600 papers, could never be criticised for being superficial or trivial. Perhaps the most characteristic feature of his taxonomic work was the way in which he took account of the animal's biology and viewed his subjects in their historical context. It is no wonder that Hubbs contributed so much to unravelling the historical biogeography of American freshwater fishes, nor that his interests should have turned to distributional problems in marine fishes as well. That, in turn, led him into oceanographical and meteorological research, while his freshwater studies took him into the fields of archaeology and human palaeoecology.

With his wife Laura, a mathematician and a dedicated companion, Carl Hubbs pioneered several studies into the genetics of fishes, in particular the application of hybridization studies to the systematics of several taxonomically "difficult" groups. Together they were responsible for discovering the now famous matroclinous and gynogenetic reproduction of the Amazon Molly, *Poecilia formosa*.

Problems of speciation and evolution, especially amongst the rich freshwater fish faunas of north and middle America, also attracted Hubbs. Interestingly, the phrase "new systematics" was first coined by him several years before it was used, apparently as a neologism, by Huxley as a title for that now classic collection of taxonomic essays he edited in 1940.

Conservation was a long standing concern of Hubbs'. He was instrumental in protecting forests, elk, fur seals, the Pacific gray whale and, after a forcefully conducted fight, the desert pupfish of Devil's Hole, Nevada.

That quite inadequate summary of Carl Hubb's research and influence on World ichthyology has overlooked his role in the development of many American research institutes and professional societies, his importance as a teacher, especially of post-graduate students, and his dedicated involvement in the more pragmatic aspects of his great love, ichthyology.

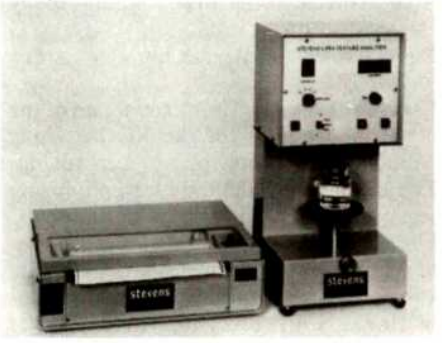
Many honours were conferred on him, including election to the National Academy of Sciences, the award of the Joseph Leidy Medal, the Shinkishi Hatai Medal of Japan, the sole honorary membership of the Japanese Ichthyological Society, and election to Foreign Membership of the Linnean Society of London.

Informally Carl Hubbs will long be remembered with warm affection by those who knew him, in particular by fellow ichthyologists for whom, irrespective of their status in the discipline, and despite his many commitments, he could always find time to share his knowledge and give his critical encouragement. Historically his contributions to ichthyology will ensure him a degree of immortality vouchsafed to few.

P.H. Greenwood

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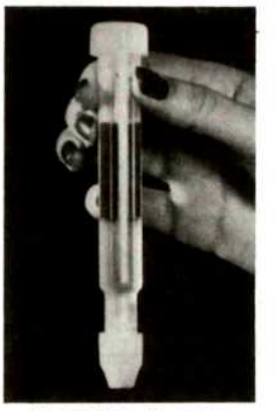
Inhalation chamber for toxicity testing. Wahmann. The 750-l inhalation chamber, specifically designed for toxicity testing through pulmonary exposure with various laboratory animal species is now available from Wahmann Manufacturing. The chamber is specifically designed to help research facilities comply with regulations of the Toxic Substance Control Act of 1977. All Wahmann Inhalation Chambers have removable caging and racking for any animal species. They are suitable for either acute or chronic studies in a broad spectrum of research testing. A free brochure with further information on Wahmann Inhalation Chambers can be obtained by circling the number shown below.
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Non-linear temperature programmer. Neslab. Non-linear temperature programs can now be performed over a range of −80°C to +150°C using Neslab constant temperature bath circulators with their new Model ETP-4RC non-linear electronic temperature programmer. This programmer will accept a signal from a computer, microprocessor or repeat cycle timer allowing practically any heat/cool configuration related to time.
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Water baths. Memmert. Anderman. The Memmert range of water baths covers sizes from 4 to 48 l capacity and the temperature range is from +25°C to +100°C. Water temperature is maintained by a liquid expansion thermostat which holds the desired temperature to within ± 0.5°C, and for high ambient temperatures a counter cooling coil is available for connection to the mains water supply. The latest waterbath, the WU 600 has the added advantage of a circulating pump incorporated into the bath housing. The pump gives a circulation of 30 l min^{-1} and is recommended if little temperature variation is required, especially with a fully loaded bath.
Circle No. 280 on Reader Enquiry Card.

Microcomputer-controlled double beam spectrophotometers. Perkin-Elmer has introduced the Models 320 and 330, the double monochromator microcomputer-controlled UV-vis and UV-vis-NIR spectrophotometer. Both the models incorporate a grating–grating double monochromator. Instrument resolution is 0.07 (UV-vis) nm and stray light is less than 0.0001% at 220 nm, providing a photometric range of 4.0 Å.
Circle No. 281 on Reader Enquiry Card.

Separating tube. Life Science Laboratories have introduced the Separatube. This device is designed to simplify solvent extraction of samples. Made from inert plastic the Separatube is capped at one end and incorporates a needle-valve at the other, allowing controlled solvent run-off. This disposable item is particularly useful for pathogen or radio-isotope work.
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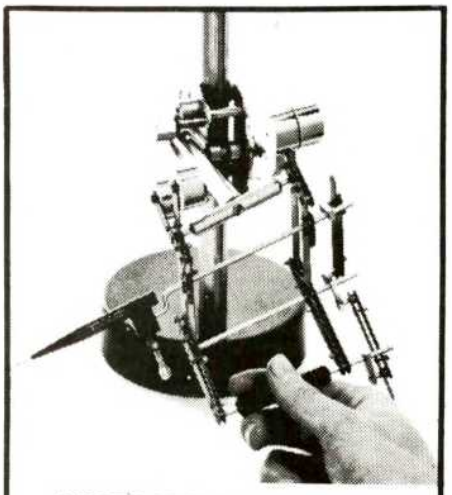


The Life Science Separatube

Multi-ingredient precision weighing system. British Aerospace Dynamics Group. Selectamix is a multi-ingredient industrial weighing control system developed by the Chiswick Division of British Aerospace Dynamics Group. Currently an eight-ingredient system is being evaluated. When a measuring sequence is initiated the Selectamix control will open the metering valve of the first ingredient until the increase in weight signalled by the load cell indicates that the correct proportion of ingredient 1 has been supplied. The first valve will then be shut and the second metering valve opened. The procedure is repeated until all eight ingredients have been measured, in sequence, into the production batch hopper in their correct proportions. Selectamix incorporates Random Access. Memory (RAM) and Erasable Programmable Read Only Memory (EPROM) facilities and is compatible with most existing computer installations.
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Orbital mixer. Wheaton Instruments has introduced an orbital mixer which will agitate the contents of large dishes, Petri dishes and slides. The speed of the multi-directional movement is steplessly adjustable over 9–45 c.p.m. The 280 × 280 mm supporting plate has a rubber surface for increased friction with the vessels. The mixer will accommodate a weight equivalent to 7 l of water.
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Mini-Ads:



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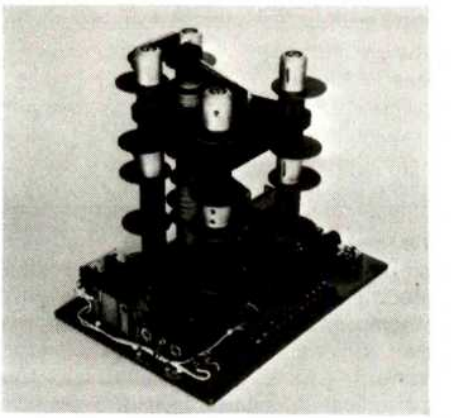
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Measurement of unbound moisture in powders and granules. Cosa Corporation. Rapid, on-site measurements of unbound moisture in powders and granules are made practical by the Michel VR2 meter with disk sensor. A small sample is simply scooped into the rugged disk sensor. Equilibrium is reached in a few seconds and the value is read from the meter's relative-humidity scale. Available from Cosa Corporation , the instrument and sensor make measurements in the range of 0.001–6% water and higher in some applications. The meter may be calibrated in equilibrium relative humidity to cover the typical range of 2 – 99% RH. Accuracy generally is 5% of scale deflection. The Model VR2 meter operates wherever it can be plugged into a 120 VAC outlet.
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Leak detector. Varian has introduced the latest model in its 936 series helium mass spectrometer leak detectors. Weighing only 25 kg, the 936-40 measures $21 \times 35 \times 50$ cm and is as portable and convenient as a small suitcase. A unique valve block design accurately positions roughing and foreline valves during the test cycle and prevents damage due to improper sequencing. The block is operated by a single handle on the system's cabinet. No liquid nitrogen is required.
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The Ross ER Series rotary relay

High-voltage rotary relay. Ross Engineering Corporation introduces the ER Series HV rotary relay, the newest addition to Ross' line of 5 kV to 300 kV PK Test high voltage relays. The ER-50 has a voltage rating of 50 kV PK Test with a continuous current capacity of less than 1 mA to 50 A or more. It is designed in a SPDT or DPDT configuration with standard actuator voltages of 115 V and 230 V a.c. (50 or 60 Hz) with other, a.c. or d.c., voltages and frequencies available. The ER Series relay can be used for HV transfer, capacitor discharge, polarity reversing, safety grounding, and tap and load selection. It is useful with a wide range of currents and has a high momentary current capability. It has fully visible contacts and is constructed using fire-retardant epoxy glass insulation.
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Glass knife holder. The introduction of the glass knife holder for the Reichert-Jung Autocut heavy duty microtome provides an inexpensive method for producing sections from plastic embedded materials. The 40-μm retraction on the return stroke of the Autocut's cutting cycle allows glass knives to be used for both dry and floated sections. Full ultra-microtome style orientation of the knife clearance angle, knife rotation in two directions and lateral movement of the holder are all built-in features. Its most important application is for the production of high quality methacrylate sections for light microscopy.
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Electroiontophoresis electrometer/transductor. W.P. Instruments. The Model 7160 electroiontophoresis/electrometer transductor from WPI features a light-weight, corrosion-resistant electronic probe allowing quiet recording in the 'current OFF' mode and any waveform, polarity or time sequence of injecting current (1 — 1,000 nA) in the 'current ON' mode. In the 'Stand-By' mode the probe tip will remain at or near zero volts until ready to use. The instrument has a voltage excursion range of ± 100 V over which the probe may swing.
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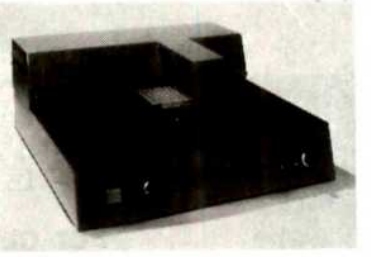
Dispenser. Alpha. A new 'mini' model in the established Millimatic Dispenser range has been introduced by Alpha Laboratories. The unit will dispense from 0.25 ml to 3 ml in 50-μl increments, repetitively, accurately and simply. Like all the other models in this range, the new Millimatic uses both major and vernier scales which lock positively into place making each volume adjustment a positive stop. The scales give a choice of over 60 different volume settings from one small unit. The reservoir is 500 ml and the unit is easily dismantled for maintenance and can be autoclaved. In addition, the 'mini' incorporates a new design of glass and ceramic valve which is now totally enclosed in a Teflon housing. A feature of particular interest is the bore of the fluid pathway which is wide enough to take fairly coarse particulate suspensions such as charcoal, kaolin and macroaggregations of albumin. This dispenser gives exceptional performance with an accuracy of better than 1% at 100 μl with even better results at the higher volume settings. It is also available as a diluter/dispenser with the same dispensing volumes and sample uptake volumes in the range 5–100 μl.
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Continuous CO_2 monitor. Beckman. The new LB-3 from Beckman, a continuously monitoring CO_2 analyser, is designed for applications in intensive care, neonatology, anaesthesia and neurosurgery. The LB-3's special, self-cleaning sampling system monitors the patient circuit for 4.5 min, then reverses gas flow through the sample lines for 30 s and cleans any accumulated moisture from the sample system. The analyser has audible and visual alarms for both high and low CO_2 levels, and a variable sample rate of 50 to 400 ml min^{-1}.
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TV Image analyser modules. Leitz announce the introduction of powerful new modules for its television-based image analysis system, TAS. A 100 gray level module scans an image or individual structures under software control for density histograms or reflectograms and returns integrated, normalised density values over a selected geometry. A module with a hardware arithmetical logic unit and binary image planes — each storing a complete threshold image — combines images at high speed. It can extract image detail, clean images, add and subtract images and set an image as a mask. This is done under the control of a comprehensive software operating system resident in a built-in microcomputer. All machine functions and image operations can be programmed in a BASIC-like language on a video terminal. The program can be stored for repetitive use.
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Magnetic hotplate stirrer. Cabburn Sterilizers. A new magnetic hotplate stirrer, which automatically reverses direction every 6s, and which has a top plate covered in white deconyl high density nylon to resist most spillages and abrasion, has been introduced by Cabburn Sterilizers. Called the Autorev-H, the unit measures 216 mm wide × 140 mm high × 210 mm deep. The 145-mm diameter hotplate is made of aluminium alloy in a natural finish, with the sheathed heating element moulded into the casting to ensure good heat transfer. The unit has a stirring capacity of up to 4 l, depending on viscosity. The Autorev-H incorporates controls including a single direction or automatic reversing illuminated switch, a speed control which is continuously variable over 100–1,450 r.p.m., an energy regulator control and a hotplate indicator light.
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MicroELISA readers. Dynatech have announced two entirely new 'through-the-plate' optical microELISA readers. A manual version capable of reading a complete ELISA plate in less than 5 min, and both print and analyse test results, and an automatic twin beam version which fully analyses and prints-out results of a complete MicroELISA plate in less than 1 min are also available.
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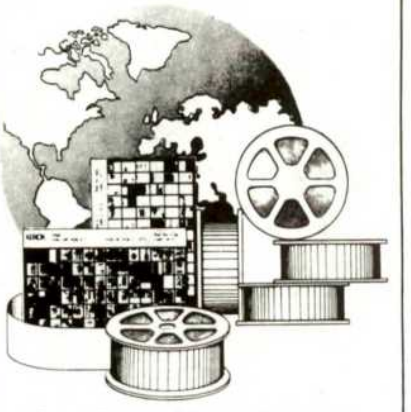


A Dynatech micro ELISA reader

Micromanipulator. Singer. The purpose of the Singer Microforge is to make easily from glass, micropipettes, needles both straight and curved, loops etc. The glass and heating element are viewed through a binocular eyepiece microscope with fine focusing. The glass and platinum heating element are held in fully adjustable and rotatable holders and the special feature of the instrument is that these holders are manipulated by two joysticks that move the work in the focal plane. This design advance makes possible, with little practice, the performance of intricate and complicated movements. The microscope and joysticks and other controls are conveniently mounted on a single rigid chassis within the instrument case. The heating element is thyristor controlled, and the element temperature is smoothly and finely adjusted by a large, conveniently placed knob.
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Crystal analyser. Siemens has developed a crystal analyser that will make a detailed structural crystal examination in about 1 min. The analyser achieves this by using a high-speed X-ray diffraction system coupled with a position-sensitive detector and associated electronics. The detector allows incident X-ray quanta to be located to within 50 μm on a high ohmic counter wire, even at relatively wide detector apertures. Consequently, the X-ray diffraction lines over a range of 5° can be recorded in a few seconds. Using the fastest goniometer speed (400° min^{-1}) a complete diffraction diagram may be recorded in a matter of minutes.
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Cryogenic stage. Cambridge Instruments. A new cryogenic stage developed by Cambridge Scientific Instruments enables biological materials in the frozen hydrated state to be continuously examined and analysed in the scanning electron microscope at a temperature such that freeze drying and ice crystal growth cannot occur. The stage package comprises a specimen stage and airlock, a liquid nitrogen cooler transfer unit, a temperature monitoring unit, a specimen chamber anti-contamination plate and a transmission detector. It is easily attached to the current Stereoscans 150 and 604, and can also be readily fitted to the Stereoscans 180, S4 and S2A. Because of its ease of fitting and removal, the stage is not limited to only low temperature scanning electron microscopy. The transfer unit incorporates an LN2 chilled 'cold garage' into which the specimen is drawn, keeping it below −150°C.
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Stock shapes. Chemplast. Chemfluor TFE stock shapes, made of Teflon and other TFE fluoropolymers, include centreless ground extruded rod, moulded and stress relieved sheet, rectangular stock, square and hexagonal rod, extruded heavy wall tubing and moulded tubes. All stock shapes are available in 12 standard colours and can be furnished with reinforcing fillers or with surfaces pretreated for adhesive bonding.
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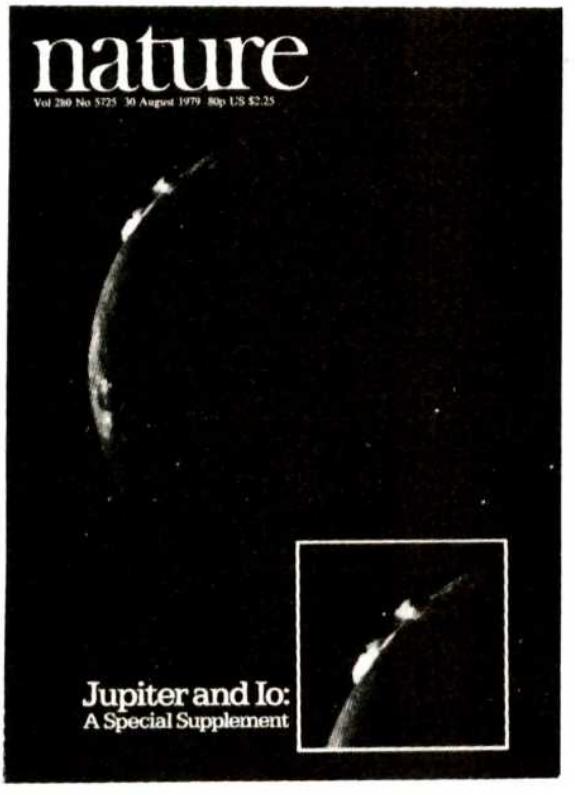
Flow measurement and sampling systems. The Manning range of trade effluent flow measurement and sampling systems comprise a range of quick insert flumes covering a flow range of up to 0.85 x 10^6 gallons per day. The flumes are used in conjunction with portable flow-metering instruments which feature 24-hr and 7-d chart recorder functions, built-in integrators and sampler output for flow proportional sampling. Flowmeters are available with dipping probes or the latest ultrasonic devices. Installation and calibration of the flowmeter/flume systems may be achieved in a matter of minutes.
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Laminair flow cabinet. Laboratory Impex. One of the few laminair flow cabinets which conform to the new BS 5726 for Microbiological Safety Cabinet, is marketed by Laboratory Impex. With the Laminair HLB 2448 an even flow of air is drawn into a fixed working aperture, to first minimise the risk of particles escaping from the working area into the laboratory. The unfiltered room air is directed immediately to the first of three HEPA filters, without encroaching on the actual working area. Filtered air is ducted to the top of the unit where the flow is divided between that ducted to exhaust (to compensate for the continuous air inflow) and that to be directed onto the working area. An important feature of the Laminair system is that in both cases the clean air is HEPA filtered a second time. An additional benefit in the use of multiple HEPA filters is that the flow of air over the working area is truly laminair. Turbulence is virtually eliminated by the use of vertical interior surfaces.
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Arc lamps. The range of compact arc lamps from Canrad-Hanovia have been designed to provide optimum stability and performance in the widest range of applications varying from graphic arts equipment to solar simulation. Mercury-xenon lamps for enhanced UV output and stability, lamps for high overall intensity from a compact arc, and diffused arc lamps for medium intensity uniform illumination, are available in sizes from 35W to 30kW.
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Applications note on two-photon spectroscopy. Chromatix. A nine-page applications note describing the use of a flashlamp pumped tunable dye laser in two-photon spectroscopy is now available from Chromatix of Sunnyvale, California.
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BARKER, F. (ed.). Trondhjemites, Dacites, and Related Rocks. (Developments in Petrology, 6.) Pp.xv + 659. ISBN-0-444-41765-6. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1979.) Dfl.135; $65.75.

BERRY, Brian J.L. (ed.). The Nature of Change in Geographical Ideas. (Perspectives in Geography, 3.) Pp.x + 167. ISBN-0-87580-063-7. (Dekalb, Ill.: Northern Illinois University Press, 1978.) $17.50.

BIOGE CHEMISTRY OF ESTUARINE SEDIMENTS. (Proceedings of a Unesco/SCOR Workshop held in Melreux, Belgium, 29 November to 3 December 1976.) Pp.293. ISBN-92-3-101594-X. (Paris: Unesco, 1978.)

BOLLE, H.J. (ed.). Remote Sounding of the Atmosphere from Space. (Proceedings of a Symposium of the Twenty-first Plenary Meeting of COSPAR, Innsbruck, Austria, 29 May to 10 June 1978.) (Advances in Space Exploration, Vol.4.) Pp.xiv + 256. ISBN-0-08-023419-4. (Oxford and New York: Pergamon Press, 1979.) £20. $40.

DEER, W.A., HOWIE, R.A., and ZUSSMAN, J. Single-Chain Silicates. (Rock-Forming Minerals, Vol.2A.) Second edition. Pp.668. ISBN-0-582-46522-2. (London: Longman Group, Ltd.; New York: Halsted Press, a Division of John Wiley and Sons, Inc., 1978.) £25.

FAIRBRIDGE, Rhodes W., and FINKL, Jnr., Charles W. (ed.). the Encyclopedia of Soil Science. Part 1: Physics, Chemistry, Biology, Fertility, and Technology. (Encyclopedia of Earth Sciences, Vol.XII.) Pp.xxi + 646. ISBN-0-87933-176-3. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1978. Distributed world wide by Academic Press, New York and London.) $59.50.

HURLBUT, Cornelius S., and SWITZER, George S. Geology. Pp.xii + 243. ISBN-0-471-42224-X. (New York and Chichester: Wiley-Interscience, John Wiley and Sons, 1979.) £13.50.

JANSEN P Ph., VAN BENDEGOM L., VAN DEN BERG J., DE VRIES M, and ZANEN A. (ed.). Principles of River Engineering. The non-Tidal Alluvial River. Pp.xv + 509. ISBN-0-273-01139-1. (London: Pitman Publishing Ltd., 1979) £45.00.

JONES, Gareth. Vegetation Productivity. (Topics in Applied Geography.) Pp.xi + 100. ISBN-0-582-48577-0. (London and New York: Longman, 1979.) £3.95 net.

LAPORTE, Leo F. Ancient Environments. (The Prentice-Hall Foundations of Earth Science Series.) Pp.163. Second edition. ISBN-0-13-036384-7. (Englewood Cliffs, NJ. and Hemel Hempstead: Prentice-Hall, 1979.) Cloth £6.45; Paperback £4.50.

McLOUGHLIN, John C. Archosauria: a New Look at the Old Dinosaur. Pp.viii + 117. ISBN-0-7139-1272-3. (London: Allen Lane, Penguin Books, Ltd., 1979.) £4.50 net.

MAGNETOSPHERIC BOUNDARY LAYERS - a Sydney Chapman Conference. (Proceedings of an International Conference held in Alpback, Austria, 11-15 June 1979) (ESA SP-148.) Pp.xvi + 411. (Paris: European Space Agency, 1979.) FF. 120. (Aug).

NITECKI, Matthew H. (ed). Mazon Creek Fossils. (Papers presented at the Mazon Creed Fossils Symposium, held in Ann Arbor, Michigan, May 1, 1978.) Pp.xiv + 581. ISBN -0-12-519650-4. (New York and London: Academic Press, 1979.) $29.50.

PROSPECTING IN AREAS OF GLACIATED TERRAIN 1979. (Papers presented at a Symposium organized by the Irish Association for Economic Geology and held in Dublin, Ireland, 26-29 August 1979.) Pp.vi + 110. ISBN-0-900488-46-8. (London: The Institution of Mining and Metallurgy, 1979.) £18.

PYLE, Gerald F. Applied Medical Geography. (Scripta Series in Geography.) Pp.xiv + 282. ISBN-0-470-26643-0. (Washington, DC.: V.H. Winston and Sons, 1979. Distributed solely by Halsted Press, a Division of John Wiley and Sons, Inc., New York and Chichester,) £12.

RIEDLER, W. (ed). Scientific Ballooning. (Proceedings of Symposium on the Scientific Use of Balloons and Related Technical Problems of the Twenty-first Plenary Meeting of COSPAR, Innsbrock, Austria, 29 Ma to 10 June 1978.) (Advances in Space Exploration, Vol.5.) Pp.xii + 220. ISBN-0-08-023420-8. (Oxford and New York: Pergamon Press, 1979.) £20; $40.

ROBERTS, Walter Orr, and LANSFORD, Henry. The Climate Mandate. Pp.viii + 197. ISBN-0-7167-1055-2. (San Francisco and Reading: W.H. Freeman and Company, 1979.) Hard £6.70; Soft £3.30.

STEEL, Rodney, and HARVEY, Anthony P. (ed). The Encyclopaedia of Prehistoric Life. Pp.218. ISBN-0-85533-175-5. (London: Mitchell Beazley Publishers, Ltd., 1979.) £12.

STOWE, Keith S. Ocean Science. Pp.xii + 610. ISBN-0-471-04261-7. (New York and Chichester: John Wiley and Sons, 1979.) £9.50.

WAHLSTROM, Ernest E. Optical Crystallography. Fifth edition. Pp.488. ISBN-0-471-04791-0. (New York and Chichester: John Wiley and Sons, 1979.) £12.

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WHITE, R.E. Introduction to the Principles and Practice of Soil Science. Pp.x + 198. ISNB-0-632-00052-X. (Oxford and London: Balckwell 1979.) £8.50.

Applied biology

STEELE, James H. (ed.). GRC Handbook Series in Zoonoses. Section A: Bacterial, Rickettsial and Mycotic Diseases, Vol.1. Section Editors: Herbert Stoenner, William Kaplan and Michael Torten. Pp.643. ISBN-0-8493-2906-X. (Boca Raton, Florida: CRC Press, Inc., 1979. Distributed in UK by Blackwell) £60.

SUNDARAM, P.V., and ECKSTEIN, F. (ed.). Theory and Practice in Affinity Techniques. Pp.x + 246. ISBN-0-12-677150-2. (London and New York: Academic Press, 1978). £10.50; $21.75.

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TAUSSIG, Michael J. Processes in Pathology: An Introduction for Students of Medicine. Pp.x + 456. ISBN-0-632-00366-9. (Oxford and London: Blackwell, 1979.) £6.50.

TUCEK, Stanislav (ed.). The Cholinergic Synapse. (Progress in Brain Research, Vol.49.) Pp.xx + 511. ISBN-0-444-80105-7. (Amsterdam, New York and Oxford Elsevier, 1979.) Dfl.190; $92.75.

WHITAKER, Haiganoosh, and WHITAKER, Harry A. (ed.). Studies in Neurolinguistics, Vol.4. (Perspectives in Neurolinguistics and Psycholinguistics.) Pp.xviii + 357. ISBN-0-12-746304-6 (v.4). (New York and London: Academic Press, 1979.) $26.

WILCKE, Harold L., HOPKINS, Daniel T., and WAGGLE, Doyle H. (ed.). Soy Protein and Human Nutrition. (Proceedings of the Keystone Conference held in Keystone, Colorado, May 22-25, 1978.) Pp.xiv + 406. ISBN-0-12-751450-3. (New York and London: Academic Press, 1979.) $25.

WISEMAN, Alan (ed.). Topics in Enzyme and Fermentation Biotechnology, 3. Pp.294. ISBN-0-85312-140-0. (Chichester: Ellis Horwood, Ltd., 1979. Distributed by Halsted Press, a Division of John Wilet and Sons, New York, Chichester, Brisbane and Toronto.) £19.50.

WINICK, Myron (ed.). Nutrition: Pre-and Postnatal Development. (Human Nutrition: a Comprehensive Treatment, Vol.1.) Pp.xx + 496. ISBN-0-306-40132-0. (New York and London: Plenum Press, 1979.) $39.50.

Anthropology

BERNSTEIN, Irwin S., and SMITH, Euclid O. (ed.). Primate Ecology and Human Origins: Ecological Influences on Social Organization. (Garland Series in Ethology.) Pp.xvii + 362. ISBN-0-8240-7080-1. (New York and London: Garland STPM Press, 1979.) $24.50.

EBIHARA, May, and GIANUTSOS, Rosamond (ed.). Papers in Anthropology and Linguistics. (Annals of the New York Academy of Sciences, Vol. 318.) Pp.114. ISBN-0-89072-077-0. (New York: New York Academy of Sciences, 1978.) $19.

ISAAC, Glynn, and LEAKEY, Richard E.F. (with an introduction by). Human Ancestors. (Readings form *Scientific American*.) Pp.vi + 130. ISBN-0-7167-1101-X. (San Francisco and Reading: W.H. Freeman and Company, 1979.) Hardcover £7.40; Softcover £2.80.

LAMBERG-KARLOVSKY, C.C. (with introductions by Hunters, Farmers, and Civilizations: Old World Archaeology. (Readings from *Scientific American*.) Pp.viii + 306. ISBN-0-7167-1074-9. (San Francisco and Reading: W.H. Freeman and Company, 1979.) Hardcover £10; Softcover £4.50.

LEAKEY, Richard, and LEWIN, Roger. People of the Lake: Man; His Origins, Nature and Future. Pp.223 + 32 photographs. ISBN-0-00-219502-X. (London: Collins, 1979.) £6.50.

NEEDHAM, Rodney. Symbolic Classification. (The Goodyear Perspectives in Anthropology Series.) Pp.xii + 78. ISBN-0-87620-873-1. (Santa Monica, CA.: Goodyear Publishing Company, Inc., 1979. Distributed in UK by Prentice-Hall, Hemel Hempstead.) Cloth £4.50; Paperback £3.20.

RENFREW, Colin, and COOKE, Kenneth L. (ed.). Transformation Mathematical Approaches to Culture Change. Pp.xxii + 515. ISBN-0-12-586050-1. (New York and London: Academic Press, 1979.) $39.50; £25.65.

SCHIFFER, Michael B. (edited by). Advance in Archaeological Method and Theory, Vol.2. Pp.ix + 340. ISBN-0-12-003102-7. (New York and London: Academic Press, 1979.) $26.

Psychology

AGRAS, W. Stewart, KAZDIN, Alan E., and WILSON, G. Terence. Behavior Therapy: Toward an Applied Clinical Science. (A Series of Books in Psychology.) Pp.x + 173. ISBN-0-7167-1087-0. (San Francisco and Reading: W.H. Freeman and Company, 1979.) Hard £7.40; Soft £3.10.

BRIERLEY, Harry. Transvestism: a Handbook with Case Studies for Psychologists, Psychiatrists and Counsellors. Pp.xii + 259. ISBN-0-08-024686-9. (Oxford and New York: Pergamon Press, 1979.) Hard £13; Felxi £6.50.

CHOROVER Stephen L. From Genesis to Genocide. The Meaning of Human Nature and the Power of Behavior Control. Pp.xiii + 238. ISBN 0-262-03068-3. (Massachusetts: M.I.T. Press, 1979). £9.75.

COOK, Mark, and WILSON, Glenn (eds). Love and Attraction: An International Conference. Pp.xvii + 554. ISBN-0-08022234-X. (Oxford and New York: Pergamon Press, 1979) £27.50.

DAS J.P., KIRBY John R., JARMAN Ronald F. (with a chapter by CUMMINS James P.) Simultaneous and Successive Cognitive Processes. Pp.247. ISBN 0-12-203150-4. (New York: Academic Press Inc. 1979). $16.00.

EYSENCK, Hans J. The Structure and Measurement of Intelligence. With contributions by David W. Fuller. Pp.v + 253. ISBN-3-540-09028-2. (Berlin and New York: Springer-Verlag, 1979.) DM 44; $24.20.

FANTINO, Edmund, and LOGAN, Cheryl A. The Experimental Analysis of Behavior: a Biological Perspective. (A Series of Books in Psychology.) Pp.xiii + 559. ISBN-0-7167-1036-6. (San Francisco and Reading; W.H. Freeman and Company, 1979.) £8.40.

GOLDSTEIN, Aronold P., HOYER, William J., and MONTI, Phillip J. (ed). Police and the Elderly. (Pergamon General Psychology Series.) Pp.x + 116. ISBN-0-08-023893-9. (Oxford and New York: Pergamon Press, 1979.) Hard £6.25; Flexi £3.50.

KESSLER, Seymour (ed.). Genetic Counselling: Psychological Dimensions. Pp.xix + 248. ISBN-0-12-405650-4. (New York and London: Academic Press, 1979.) $18.

MACVAUGH, Gilbert S. Frigidity: What You Should Know About Its Cure with Hypnosis. Pp.xix + 369. ISBN-0-08-021748-6. Oxford and New York: Pergamon Press, 1979.) £20.

PHYE, Gary D., and RESCHLY, Daniel J. (ed.). School Psychology: Perspective and Issues. (Educational Psychology.) Pp.xiii + 348. ISBN-0-12-554250-X. (New York and London: Academic 1979.) $22.

POPE, Benjamin. The Mental Health Interview/Research and Application. (Pergamon General Psychology Series.) Pp.xv + 541. ISBN-0-08-019383-0. (Oxford and New York: Pergamon Press, 1979.) £14.25.

POWELL, Graham E. Brain and Personality. Pp.vii + 113. ISBN-0-566-00272-8. (Farnborough, Hants.: Saxon House, Teakfield Limited, 1979.) £8.50.

REESE. Hayne W., and LIPSITT, Lewis P. (ed.). Advances in Child Development and Behavior, Vol.13. Pp.xii + 313. ISBN-0-12-009713-3. (New York and London: Academic Press, 1979.) $24.

RIEGEL, Klaus F. Foundations of Dialectical Psychology. Pp.xv + 199. ISBN-0-12-588080-4. (New York and London: Academic Press, a Subsidiary of Harcourt brace Jovanovich, Publishers, 1979.) $15.

RODEWALD, H. Keith. Stimulus Control of Behavior. Pp.vii + 162. ISBN-0-8391-1447-8; (Baltimore, Md.: University Park Press, 1979. Distributed in the UK by MTP Press, Ltd., International Medical Publishers, Lancaster.) £6.25.

ROMMETVEIT R. and BLAKAR R. M. (ed.). Studies of Language, Thought and Verbal Communication. Pp.466. ISBN 0-12-594660-0. (London: Academic Press Inc. (London) Ltd. 1979.) $22.80.

STEWART, Robert A., POWELL, Graham E., and CHETWYND, S. Jane. Person Perception and Stereotyping. Pp.ix + 318. ISBN-0-566-00072-5. (Farnborough, Hants: Saxon House, Teakfield, Ltd., 1979.) £9.50.

STEINAKER, Norman W., and BELL, M. Robert. The Experiential Taxonomy: a New Approach to Teaching and Learning. (Educational Psychology.) Pp.xvi + 198. ISBN-0-12-665550-2. (New York and London: Academic Press, 1979.) $15.

SPRAGUE, James M., and EPSTEIN, Alan N (ed.). Progress in Psychobiology and Physiological Psychology. Pp.xiv + 353. ISBN -0-12-542108-7. (New York and London: Academic Press, 1979.) $35.

UTTLEY, A.M. Information Transmission in the Nervous System. Pp.xiv + 11[illegible] ISBN-0-12-709750-3. (London and New York: Academic Press, 1979.) £7.50; $15.50.

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APPOINTMENTS VACANT

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
BONE & JOINT RESEARCH UNIT
LABORATORY SCIENTIFIC OFFICER

Required to assist in project involving production of monoclonal antibodies and research into the cause of rheumatic diseases. Knowledge of sterile techniques useful but not essential. The appointment is funded by The Arthritis and Rheumatism Council for an initial period of one year. Salary £3,888 plus £354 London Weighting.

For further details please telephone Miss McCall 01-247 8939 or apply in writing, enclosing full c.v., to Professor E.J. Holborow, Bone and Joint Research Unit, The London Hospital Medical College, Turner Street, London E1 2AD, quoting reference BJRU/11/79. 3647(A)

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY
BACTERIOLOGIST

A bacteriologist is required to assist in the preparation of demonstrations and class material for the course in medical microbiology. Duties include assisting the lecturing staff and participation in research. Candidates should have a degree in microbiology or qualification as a technician, preferably with experience in medical microbiology.

Salary is expected to be in the £4,000 to £5,000 range depending on the qualifications and experience of the successful applicant.

Applications with curriculum vitae and names of two referees should be sent to Dr H.H. Johnston, Bacteriology Department, Level 7, John Radcliffe Hospital, Headington, Oxford. 3641(A)

ST. MARY'S HOSPITAL MEDICAL SCHOOL
DEPARTMENT OF VIROLOGY
Praed Street, London W2 1PG

A vacancy exists for a Junior Medical Laboratory Scientific Officer in the diagnostic section of this Department, which provides a District service. Candidates must have a minimum of 4 appropriate 'O' levels or preferably an ONC or science degree and must be prepared to follow the relevant courses of study leading to state registration as a Medical Laboratory Scientific Officer. Salary on a Whitley Council scale from £2,235 p.a. to £3,375 p.a. or from £2,535 to £3,501, depending on age and qualifications, plus London Weighting.

Further information from Mr C Kenny, Chief MLSO, tel 01-723 1252 ext 200. Applications should be sent to the School Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG. 3598(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF NEUROLOGICAL STUDIES
RESEARCH ASSISTANT

Applications are invited from graduates for the post of Research Assistant to assist in a variety of laboratory projects connected with research in cerebrovascular disease (strokes). The post is available for two years.

Salary, according to age and experience, within the range £4,515 to £5,649 per annum (including £740 per annum London Allowance).

Applications, including curriculum vitae and the names and addresses of two referees should be submitted to Dr M J G Harrison, Institute of Neurological Studies, Sir Jules Thorn Institute, The Middlesex Hospital Medical School, London W1. 3600(A)

ASSISTANT REGISTRATION MANAGER

We are a major international company manufacturing ethical pharmaceuticals. The Company has been successful in recent years in identifying a series of valuable new drugs and has a continuing research programme involving further potential new drugs.
The Registration group is a small team of graduates whose principal function is the organisation of scientific data for presentation to the Committee on Safety of Medicines. The group works closely with the Scientific Information group.
The post of Assistant Manager in this department is now vacant. Candidates should be graduates in a biological science and should already have some experience of product registration or scientific information in the pharmaceutical industry. They should be familiar with the requirements of the Medicines Act. Facility in organisation of scientific data and the ability to write clearly and concisely on scientific topics are essential.
There is a possibility that a candidate with less experience could be appointed at a lower level.

Please apply to: Mrs J C M Andrews, Personnel Officer, Wyeth Laboratories, Huntercombe Lane South, Taplow, Maidenhead, Berkshire. SL6 OPH. Telephone Number: Slough 28311.

Wyeth Laboratories 3650(A)

UNIVERSITY OF LEICESTER
DEPARTMENT OF CHEMISTRY

Applications are invited for a
RESEARCH ASSOCIATE
to work with Professor M.C.R. Symons on various electron spin resonance projects of biological significance, for two years. Salary on scale £4,333 to £7,521.

Please contact Professor M.C.R. Symons, Head of Department of Chemistry, University of Leicester, Leicester LE1 7RH, as soon as possible. 3620(A)

UNIVERSITIES OF BRISTOL AND MANCHESTER
DARESBURY LABORATORY
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a postdoctoral research associate to coordinate an SRC collaborative computational project on molecular collisions. The project will involve the development of computer programmes for use at Daresbury and other centres. The post is based either at the Department of Chemistry, University of Manchester under Dr J.N.L. Connor or at the Department of Chemistry, University of Bristol, under Dr G.G. Balint Kurti or Dr J. Gerratt.

The location of the post will depend on the field of interest of the successful candidate. The project will be carried out in collaboration with Professor P.G. Burke's group at Daresbury Laboratory.

Salary within the range £4,333 to £5,199 pa. Superannuation.

Applications with curriculum vitae and the names of two referees to Dr J.N.L. Connor, Department of Chemistry, University of Manchester, Manchester M13 9PL by December 1st, 1979. 3583(A)

STRANGEWAYS RESEARCH LABORATORY
RESEARCH ASSISTANT

required to work as part of a small research team, on proteinases and proteinase inhibitors. Applicants should have a good Honours Degree, or H.N.C., preferably with several years experience of practical work in a biochemical laboratory. The post is for 3 years, with a starting salary in the range £3,768 to £4,232.

The successful applicant may have the opportunity to work for a higher degree.

Applications to Dr A.J. Barrett, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN. 3616(A)

STANFORD UNIVERSITY
GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open but we are particularly interested in creative young person, with a well established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

Scientific Careers on the fringe of the Lakes

We are responsible for the UK manufacture of Glaxo pharmaceuticals and their sale in this country.

Our Ulverston factory, involved in the production of bulk pharmaceutical products, incorporates one of Europe's largest fermentation works and is located in the southern part of the Lake District. The area is renowned for its natural unspoilt beauty and offers a wide range of recreational amenities.

Microbiologist

required in the Strain Selection Unit of Technical Services to join a multi-disciplinary team responsible for the selection and development of improved strains for production fermentations, including selection and screening techniques and inoculum development routes.

You should have a sound knowledge of microbial physiology, biochemistry and genetics, preferably with relevant postgraduate experience. An appreciation of statistical analysis advantageous. Close collaboration with multi-disciplinary scientific staff and liaison with University and research institutes will also be involved.

Fermentation Scientists

The Fermentation Pilot Plant Unit is responsible for development work on fermentation products on both pilot and main plant scale, optimisation of new strains, process modifications and new fermentation techniques.

One of the two appointments is an opportunity for a PhD or a good honours BSc in biological sciences, to lead one of the development teams. You should have three to five years' experience in fermentation technology, including team management. Good interpersonal skills and a proven record of implementing new ideas are also required.

The second appointment is in a development team and you should have a minimum of a good honours degree in biological sciences, and preferably some experience in fermentation.

For all appointments competitive salaries will be paid, together with the opportunity to participate in Glaxo Group profitability, and relocation expenses, where appropriate, to this very pleasant part of Cumbria.

Please write or telephone for an application form to: The Personnel Officer (RDN), Glaxo Operations UK Limited, North Lonsdale Road, Ulverston, Cumbria LA12 9DR. Tel: Ulverston 52261, ext. 92.

3645(A)

GLAXO OPERATIONS

THE UNIVERSITY OF ALBERTA
DEPARTMENT OF GEOLOGY
Edmonton, Alberta
Canada T6G 2E3

pplications are invited for a full-time ntinuing faculty position in the field of deposits/economic geology from uly 1980. Suitable applicants may have erience in any aspect of the mineralogy, rology or geochemistry of ores. The partment is exceptionally well equipped teaching and research in these areas of dy. The level of the appointment is n: the current base of the scale is ,696 plus fringe benefits.
pplications, with names of three rees, should be sent to the Chairman of Department, R. St J. Lambert, from m further details may be obtained. sing date 15 January, 1980. The iversity of Alberta is an equal-portunity employer. W491(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF MEDICINE
POST-DOCTORAL IMMUNOLOGIST

required for work on the role of complement in induction of immunological responses. The project is supported by the Medical Research Council until October 1980 but there will be a continuing opportunity in the Unit for a suitable individual. For further details please contact Dr M.B. Pepys, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS or telephone 743 2030 extension 258.

Salary up to £5199 plus £740 London Allowance.

Application forms are available from and should be returned to the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 2/220.

3540(A)

LEEDS AREA HEALTH AUTHORITY (TEACHING)
EASTERN DISTRICT

ST JAMES'S UNIVERSITY HOSPITAL

Scientific Officer

Applications are invited from graduates with an Honours degree in one of the Biological Sciences for the post of Scientific Officer in the Regional Cytogenetic Unit established at this hospital, undertaking investigations required for patients within the Yorkshire Regional Health Authority.

Previous experience in cytogenetics is desirable, but not essential.

Application forms and job descriptions available from the Personnel Office — General Sector, Gledhow Wing, St James's University Hospital, Beckett Street, Leeds LS9 7TF. Tel: Leeds (0532) 33144, ext. 5103. 3608(A)

Pharmacologists

Bronchorespiratory Research

Roussel Laboratories Limited is one of the leading pharmaceutical companies in the UK. A vital part of the international Roussel-Uclaf organisation, it is engaged in the research development, manufacture and distribution of a range of important ethical pharmaceutical and allied products for use throughout the world.

One important area of current research is in new therapies for bronchial disease and we are looking for two suitably qualified pharmacologists to join an established team working in this field.

Recruitment will be at both PhD and first degree level. Candidates for the first post should have recently obtained or expect to obtain a PhD in a suitable branch of Pharmacology and be interested in the respiratory system and its diseases. Exceptionally, experienced graduates with a proven record of innovation will be considered. The second position will be of interest to graduates with a good first degree in Pharmacology, Physiology or a related science, preferably with some relevant experience.

The salary is competitive and benefits include free BUPA and life insurance, a contributory pension scheme and generous assistance with relocation expenses where appropriate.

Application should be made on our standard application form available from Rosemary Curtis, Assistant Personnel Manager, Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wilts SN3 5BZ. Tel: Swindon (0793) 24411.

ROUSSEL

3651(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH ASSISTANT

DEPARTMENT OF BIOCHEMISTRY

A research assistant (preferably with PhD) is required to investigate the immunoglobins and viral antibodies in sera from psychiatric patients. Experience in virology and/or enzyme immunoassay techniques would be an advantage.

The appointment will be made as soon as possible and will be until the 31st July, 1980 in the first instance with a possibility of renewal for two further years.

Salary scale: £4,335 to £5,778, initial placing depending on age, qualifications and experience.

Applications should be made to the Personnel Officer, Queen's University, Belfast BT7 1NN, Northern Ireland and should include the names and addresses of two referees. Closing date: 23 November, 1979. 3630(A)

ANGLO-EUROPEAN COLLEGE OF CHIROPRACTIC LTD

LECTURER

Applications are invited for the post of lecturer in Pathology, Microbiology and Haematology, as from 1st January, 1980.

The appointed person will be required to be responsible for the running of a small Clinical Haematology Laboratory in the College Clinic.

Applications together with curriculum vitae and the names of three referees to be sent to the Dean, Anglo-European College of Chiropractic, 1 Cavendish Road, Bournemouth, Dorset, BH1 1QX, as soon as possible. 3642(A)

UNIVERSITY OF PAPUA NEW GUINEA
(Port Moresby)

Applications are invited for the post of

CHIEF TECHNICIAN
in the
DEPARTMENT OF AGRICULTURE

Applicants should have a recognised qualification in Laboratory Technology, and experience relevant to laboratory work in an academic institution. Preference will be given to applicants with experience in agricultural or biological sciences.

The appointee will be responsible for the organization and administration of teaching and research laboratories of the department and for the provision of full technical services to teaching and research staff. In addition, the appointee will be required to design and implement an active training programme for National Technical staff. Duties will also include overall supervision of budgeting, purchasing of equipment, and control of stores and supplies.

Salary: K11,875 pa, (£1 sterling = K1.52). Family passages; baggage allowance; 3 year contract; 24% gratuity of which 5% is taxable; rent-free accommodation; overseas leave; education allowances; medical scheme. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Assistant Secretary, Box 4820, University PO, Papua New Guinea by 14 December 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 3604(A)

BIOCHEMIST/ CELL BIOLOGIST

To conduct studies on various aspects of the synthesis, processing and secretion of proteins. Government/University laboratory. Academic appointment possible. Initial appointment 2 years.

Send resumé and research interests to Dr David V. Cohn, VA Medical Center, Kansas City, MO 64128. Equal opportunity employer. W499(A)

UNIVERSITY OF LIVERPOOL

POST-DOCTORAL BIOCHEMIST:

CONNECTIVE TISSUE RESEARCH

The Department of Veterinary Clinical Studies is to make an experimental study, over five years, of the changes in the biochemical structure of the uterine cervix at the time of birth in animals.

Applications are invited from candidates aged not more than 30 years, with a PhD, in an appropriate discipline and preferably with experience or interest in connective tissue biochemistry, to lead the investigation of the interaction of collagen with proteoglycans in cervical tissue.

The post is tenable as soon as possible, for three years, and may be extended for a further two years.

The initial salary will be within the range £4,333 to £6,355 per annum on a scale rising to £7,521 per annum.

Applications, together with the names of three referees, should be received not later than 29th November, 1979, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/827/N. 3638(A)

INDIANA UNIVERSITY

DEPARTMENT OF CHEMISTRY

FACULTY POSITION

The Chemistry Department anticipates having a tenure-track position in Biochemistry. We are looking for a Biochemist with an interest in structure-function relationships with emphasis on the application of sophisticated contemporary tools to biological molecules or systems.

This position will require the ability to organize and present, effectively, courses at both the graduate and undergraduate levels. Moreover, potential candidates are expected to have a solid record of accomplishment in research and to show promise of developing vigorous, independent programs of scholarly investigation.

To apply, submit a brief biographical sketch, a publication list, names of three references, and a short resume of ideas for the development of teaching and research programs. Deadline for applications is December 1, 1979. Please apply to:

Dr Adam Allerhand, Chairperson, Department of Chemistry, Indiana University, Bloomington, Indiana 47405.

Indiana University is an Equal Opportunity/Affirmative Action Employer. W489(A)

POPULATION/ QUANTITATIVE GENETICIST

THE DEPARTMENT OF MEDICAL GENETICS

THE UNIVERSITY OF BRITISH COLUMBIA

Canada

Invites applications for a faculty appointment from individuals with demonstrated excellence in research in the field of population or quantitative genetics. Candidates should be capable of developing and teaching courses in population genetics.

The position is available July 1st, 1980. Members of this department act as consultants to the Health Surveillance Registry of British Columbia and, with the division of vital statistics of the province, have a record linkage project which together provide a unique data base for research.

Applicants should send an up-to-date curriculum vitae and names of three references, by January 1st, 1980, to Dr P A Baird, Dept of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC, V6T 1W5, Canada. The University of British Columbia offers equal opportunity for employment to qualified male and female candidates. W485(A)

MISSISSIPPI STATE UNIVERSITY

DEPARTMENT OF ENTOMOLOGY

ASSOCIATE FULL PROFESSOR

Applications are invited for a full time (1 mos.) appointment with rank of Associate or Full Professor in the Department of Entomology, Mississippi State University. The successful candidate will be responsible for research in pest management on field crop systems with particular emphasis on cotton and soybean insect problems, and for participation in ongoing interdisciplinary research to develop and apply insect-crop pest models and management systems. Teaching and guidance of graduate research will be expected in areas of insect ecology and/or population dynamics. PhD in entomology or relevant life science and experience in field crop research are required.

Experience in modelling and computer programming is desirable. Salary is negotiable and competitive. Application deadline: December 15, 1979. Job available January 1, 1980.

Send resume, list of publications and letters of reference to: Dr E.P. Pieters, Chairman, Search Comm., Entomology Department, Drawer EM, Mississippi State, MS 39762. Mississippi State University is an Affirmative Action Equal Opportunity Employer. W474(A)

THE UNIVERSITY OF PAPUA NEW GUINEA
(Port Moresby)

Applications are invited for the post of

LECTURER IN PHYSICAL GEOGRAPHY

to teach at introductory and advanced levels. Applicants should have a higher degree in a relevant discipline. Preference will be given to candidates with an interest in tropical land use and resource evaluation. The appointee will be required to contribute to courses in physical geography and man/land relationships. Ability to assist in courses in geographical techniques, and some experience in aerial photograph interpretation, are desirable. Tropical experience will be an advantage. There are opportunities for fieldwork funded by the University and by Government departments. The department has access to 4-wheel drive vehicles in the Port Moresby area and also along the Highlands Highway; a boat for nearshore work and laboratory facilities. Salary scale: Lecturer K11450 to 13300 pa (£1 sterling = K1.52). Family passages; baggage allowance; 3 year contract; 24% gratuity of which 5% is taxable; research support; rent-free accommodation; overseas leave; education allowances; medical scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Secretary, Box 4820, University PO, Papua New Guinea by 30 November 1979. Applicants resident in the UK should also send one application to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 3601(A)

UNIVERSITY OF AUCKLAND
New Zealand

COMPUTER SCIENCE
SENIOR LECTURESHIP/ ASSOCIATE PROFESSORSHIP

Closing date 1 February 1980. Applicants for this position in the newly formed Department of Computer Science should be suitably qualified with a higher degree in Computer Science and have had considerable research experience. The person appointed will be expected to develop courses at both undergraduate and graduate levels and to participate actively in research.

GEOLOGY LECTURESHIP

Closing date 30 November 1979. Applicants should have a postgraduate degree in igneous petrology with a background in thermodynamics and the geochemistry of igneous rocks; some preference will be given to applicants with experience of the igneous associations of island arcs and continental margins of Pacific type.

Commencing salaries within the appropriate scale will be determined in accordance with qualifications and experience. Present salary scales are:

Lecturers: NZ$13,722 to $16,861 p.a. Senior Lecturers: NZ$17,285 to $19,780 p.a. Associate-Professors: NZ$24,879 p.a.

Conditions of appointment and method of application for all positions are available from the Assistant Registrar (Academic Appointments), University of Auckland, New Zealand or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on the dates stated. 3602(A)

PROFESSOR OF ZOOLOGY

The Department of Zoology at the University of Florida announces a position, starting September 1980, for a creative and productive scholar to be appointed as PROFESSOR OF ZOOLOGY. The responsibilities of this position will be to bring an active research program to the university, to teach a graduate course in his/her field, and to make a limited, but quality, contribution to undergraduate teaching. It is expected that the research interests of the successful candidate will fall within *comparative, ecological,* or *evolutionary* zoology. There is a preference for an appointment in the fields of the genetic aspects of evolution or the physiological ecology of aquatic invertebrates, but above all the appointment must meet the primal consideration of quality.

The salary will be commensurate with the rank, experience, and reputation of the successful candidate.

Direct inquiries and submit curriculum vitae, a statement of research interests, and the names of three referees to:

Brian K. McNab
Chairman, Professor Search Committee
Department of Zoology
University of Florida
Gainesville, Florida 32611
USA
(904/392-1178)

The deadline for applications is 1 January 1980.

An Equal Opportunity/Affirmative Action Employer. W498(A)

MEDICAL RESEARCH COUNCIL
MRC PNEUMOCONIOSIS UNIT
Llandough Hospital, Penarth, South Glamorgan, CF6 1XW

CELL BIOLOGIST — RESEARCH OFFICER

A graduate or equivalent (M.I.Biol., HND, HNC etc.,) is required in the Cell Biology Section, which is currently engaged in several studies on the cellular effects of pathogenic dusts.

Essential requirements are a good knowledge of Biochemistry and at least two years post qualification experience, which should include the use of sterile techniques. Experience in tissue culture would be advantageous.

The appointed person will be responsible to a member of the scientific staff but will be expected to assist in running the section and to conduct a certain amount of independent research.

The appointment will be made on the scale £4,134 to £5,595 (£4,614 to £6,102 from 1 January, 1980), depending upon experience.

Application forms and further details obtainable from the Assistant Administrative Officer. Please quote ref. 79/10. 3619(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellows
Division of Environmental Mechanics
Canberra

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

GENERAL: Research in the Division is centred on processes of heat and mass transfer in the natural environment. Transport of energy, water, salts and gases in the soil-plant-atmosphere system are explored. The Division has 26 staff members at present including ten research scientists, working in the areas of soil physics, solar radiation, fluid mechanics, micrometeorology, and physical aspects of plant physiology. The Division is well equipped and has facilities for computing, mobile laboratories for study of the field environment, and an experimental field site located 45 km from Canberra.

Field: PHYSICS OF FERTILIZER USE — POSITION NO. 416/039

Duties: The Division's Physical Ecology Programme is investigating the exchange of gaseous nitrogen compounds between soils, plants and the atmosphere. This research is being broadened to embrace various physical aspects of fertilizer use: the efficacies of fertilizer placement; the transport of nutrients in soils by leaching and dispersion; nutrient absorption by plants and soils; the effects of the soil microclimate on microbiological activity and chemical transformations in soils. The work aims at improving agronomic techniques for fertilizer use.

The appointee will investigate, through theory and experiment, physical processes involved in the transport, absorption and loss of fertilizers applied to the soil.

Field: SOIL PHYSICS AND FIELD HETEROGENEITY — POSITION No. 416/040

Duties: The Division's Soil Physics Programme is concentrating on studies of the interaction between water and soil. The appointee will work within this framework, in a study of the variability of field soils and its consequences in terms of the field application of the theory of infiltration and redistribution of water in soil.

The following conditions apply to both positions:

QUALIFICATIONS: A PhD degree in an appropriate experimental field, a capacity for independent research, and an ability to apply the approaches and methods of physics and mathematics.

SALARY: Research Scientist: $A15,916 to $A19,509 pa. In exceptional circumstances an appointment may be offered at a higher level of salary.

TENURE: A fixed term appointment of three years is envisaged.

Applications **IN DUPLICATE**, stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and **QUOTING THE APPROPRIATE REFERENCE NUMBER(S)** should reach:-

The Personnel Officer
Australian Scientific Liaison Office
Canberra House
Maltravers Street
LONDON
WC2R 3EH

by **30th November 1979**

Applications in U.S.A. and Canada should be sent to:-

The Counsellor (Scientific)
Embassy of Australia
1601 Massachusetts Avenue N.W.
WASHINGTON D.C. 20036 3613(A)

YEAST GENETICIST
The Brewing Research Foundation

has embarked recently on the development of recombinant DNA techniques for the genetic improvement of commercially important strains of yeast. A yeast geneticist is now required to join this project.

Candidates should recently have obtained (or expect to obtain shortly) a PhD; experience (preferably with *Saccharomyces*) in the construction and genetic analysis of mutant and recombinant strains is essential. Previous work on the inheritance of cytoplasmic genetic determinants and an interest in the regulation of gene expression would be advantageous. Appointments will be at an appropriate point between £4,600 and £5,600.

Applications, including curriculum vitae, and names of two referees should be sent to: The Assistant Director, Brewing Research Foundation, Lyttel Hall, Nutfield, REDHILL, Surrey RH1 4HY. 3633(A)

ASSISTANT KEEPER NATURAL HISTORY

Edinburgh

. . . to be concerned with the curation of the extensive insect collections. Responsibilities will include detailed revisional work, identifying specimens and registering accessions, dealing with enquiries from specialists and the public, maintaining data retrieval systems and planning exhibitions.

Candidates should normally have a degree with 1st or 2nd class honours or an equivalent or higher qualification in zoology and a good knowledge of the British species of at least one major insect order (preferably Coleoptera, Diptera or Lepidoptera). Knowledge of languages, especially French and German, and experience in field preparation work advantageous.

Salary: (from 1.1.80) as AK I £6650 — £11175; or AK II £4200 — £5950. Level of appointment and starting salary according to age, qualifications and experience. Non-contributory pension scheme.

For further details and an application form (to be returned by 22 November 1979) write to Royal Scottish Museum, Chambers Street, Edinburgh, EH1 1JF. Please quote ref: G(51)382.

ROYAL SCOTTISH MUSEUM

3655(A)

UNIVERSITY OF BATH

Applications are invited from suitably qualified candidates for the post of

LECTURER IN PHYSICAL CHEMISTRY

in the School of Chemistry. Preference will be given to applicants with research experience in heterogeneous catalysis or solid state chemistry.

Salary range £4,333 to £8,992 per annum.

Application forms and further particulars are available from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 79/234. Closing date 30th November 1979.

3622(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

SENIOR LECTURER (Hon. Principal Grade)

Physicist required to provide Radiotherapy Physics services and carry out development/research work and teaching in that subject. EMI-plan and other new techniques coming.

Salary on Senior Lecturer scale £8,698 to £10,775 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 30 November, 1979. 3632(A)

Brent and Harrow Area Health Authority
Brent Health District
CENTRAL MIDDLESEX HOSPITAL,
Acton Lane, London NW10

Research Assistant

Department of Gastroenterology

A Research Assistant is required to work on a Research Programme concerned with clinical nutrition. The work is concerned with investigating the pathophysiology of diarrhoea that occurs during Enteral Nutrition.

The post is for two years at an initial salary of £3,888 plus £354 London Weighting Allowance.

It is hoped that the appointee will register for a higher degree.

Applications with curriculum vitae should be sent to Dr D.B.A. Silk, Department of Gastroenterology, Central Middlesex Hospital, London, NW10.

3637(A)

UNIVERSITY OF HONG KONG

LECTURESHIPS/ ASSISTANT LECTURESHIPS IN ANATOMY

Applications are invited for appointment from 1 January 1980 to two posts of Lecturer/Assistant Lecturer in Anatomy. Applicants should be either dental, medical or science graduates with teaching experience in the subject. The appointments carry responsibility for teaching pre-clinical medical or dental students. The appointees will be expected to engage in research work.

Annual salaries (superannuable) are: Lecturer HK$67,920 × 4,560 — 77,040 BAR 81,600 × 4,560 — 113,520; Assistant Lecturer HK$49,500 × 4,620 — 63,360. (£1 = HK$10.70 approx).

Starting salary will depend on qualifications and experience. A medically qualified appointee who has completed his pre-registration year will be appointed as a Lecturer at the minimum point of the scale.

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Recruitment Section, Secretary's Office, University of Hong Kong, Hong Kong. The closing date for applications is 30 November, 1979.

3629(A)

RESEARCH ASSISTANT

Postgraduate Research Biochemist/Physiologist/Pharmacologist required to join a research team investigating various aspects of cardiac biochemistry and pharmacology. Experience in spectrophotometry, tissue metabolite analysis or isolated tissue perfusion would be advantageous. The research unit is located in the new Rayne Institute and appointments will be made through St Thomas's Hospital Medical School.

Applications together with a curriculum vitae should be sent to Dr D J Hearse, The Rayne Institute, St Thomas' Hospital, London SE1. 3550(A)

UNIVERSITY OF REGINA

DEPARTMENT OF BIOLOGY AND CHEMISTRY

POSTDOCTORAL POSITIONS

A two year postdoctoral position (salary approx. $14,500) is available in a molecular biology-biochemistry laboratory (headed by Dr H. Bertrand) studying the genetic control of the biogenesis of mitochondrial respiratory complexes in *Neurospora crassa*. The laboratory is well equipped for research on the assembly and function of submitochondrial complexes, restriction mapping of DNA, protein and nucleic and chemistry and for genetic manipulations.

A second postdoctoral position ($1,000 Cdn/month; ending June 30, 1979 with the possibility of an additional year pending budgetary approval) is available for research on the regulation of pyrimidine and arginine metabolism in *Salmonella typhimurium* (headed by Dr R.A. Kelln). Research activities will include mutant isolation, genetic mapping, nucleotide pool quantitation enzyme assays and protein and nucleic acid chemistry.

Interested individuals should be available as soon as possible and should have completed a PhD degree in biochemistry, genetics or physiology.

Applications, in the form of a curriculum vitae with names and addresses of three references should be sent to:

Department of Biology (or Chemistry), University of Regina, Regina, Saskatchewan, Canada S4S 0A2.

W487(A)

INVERTEBRATE ZOOLOGIST

A beginning

ASSISTANT PROFESSOR or INSTRUCTOR

position

is available July 1, 1980. Candidates must have Ph.D., potential for excellence in teaching and ability to develop an independently funded, high-quality research program. Teaching responsibilities include an introductory invertebrate zoology course with laboratory and field trips, an upper level course in the applicant's speciality, and participation in the Aquatic Biology Program. Research should be field oriented and focused at the organismal, population or community level — preferably with marine organisms. We prefer someone who will interact productively with faculty in such areas as physiology, ecology, ethology and evolutionary biology.

Applicants should submit a resume including research and teaching experience, future research plans, and a list of publications; and have three letters of reference sent by January 15, 1980 to: Dr J K Waage, Division of Biology and Medicine, Brown University, Providence, RI 02912.

An Equal Opportunity/Affirmative Action Employer. W500(A)

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

to work on experimental neuropathies employing freeze-fracture techniques. The post will be held jointly between the Department of Neurological Science at the Royal Free Hospital, Hampstead, and the Department of Anatomy, St. Mary's Hospital Medical School.

Applicants, who should have previous experience in electron microscopy, are requested to send a curriculum vitae and the names of two referees by 16 November, 1979 to Professor P.K. Thomas, Royal Free Hospital, Pond Street, London NW3 2QC, from whom further particulars may be obtained. 3646(A)

UNIVERSITY OF NATAL

DEPARTMENT OF CHEMISTRY

Durban

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin for appointment to the post of

SENIOR LECTURER/ LECTURER

in Physical, Analytical or Inorganic Chemistry.

Salary in the range:

Senior Lecturer	R11 400 — R15 600 per annum
Lecturer	R 8 100 — R13 200 per annum

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, 4001, Republic of South Africa, with whom applications on the prescribed form, must be lodged not later than 15th January 1980, quoting reference Adv. D83/79. W483(A)

Consultant in Applied Statistics

Amsterdam

c. £10,000 UK equivalent

This is a new appointment within the sizeable health care subsidiary of a major multinational, and will be based at the company's research headquarters near Amsterdam. Because of the increasing complexity of new product innovation and governmental requirements, there is an important need for an experienced Statistician to join a small and highly skilled team of internal statistical consultants.

The largest proportion of consultancy requests is concerned with human health problems. The emphasis will be on experimental design, study monitoring and data analysis—with the aid of an IBM 370 computer—and there will also be opportunity and the requirement to keep abreast of current applied statistical methodology. The span of the work will be broad, and a Consultant could expect to be involved in projects ranging from toxicology studies and clinical trial design on the one hand, to chemical development problems on the other.

Because of the technical demands of this appointment, the man or woman we seek must have mathematical/statistical qualifications to at least MSc level, combined with a minimum of 3 years' applied consultancy experience and an intelligent, realistic approach to practical problems. The ability to speak Dutch is not called for as English is widely spoken in the Company. However, good opportunity and facilities to learn the language will be provided.

The starting remuneration level will depend upon experience but, as an indicator, it should provide a standard of living broadly equivalent to some £10,000 in the UK. Generous removal expenses will be paid, including provision of a substantial tax-free allowance to help settling into a new home.

Please write in confidence for an application form, or phone if you would like to discuss the appointment further before applying.

Talentmark
Leaders in Health Care Recruitment

James A Edwards, Talentmark Limited,
King House, 5-11 Westbourne Grove,
London W2 4UA. 01-229 2266 (24 hours)

3644(A)

THAMES POLYTECHNIC
SCHOOL OF MATERIALS SCIENCE AND PHYSICS
RESEARCH ASSISTANT

A Research Assistant is required in the Division of Physics to investigate the properties of amorphous semiconductors. The successful applicant will have a good honours degree in physics or electronic engineering and will register for a higher degree. Salary scale £3,321 to £3,705 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by 13 November, 1979. 3609(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL
(University of London)
DEPARTMENT OF BIOCHEMISTRY

Applications are invited from graduates in Science or Medicine for a research post funded by the Cancer Research Campaign to join a small group working on the possible use of lipid vesicles (liposomes) in cancer treatment. Experience in the field of tissue culture would be an advantage. Excellent opportunities exist for collaborative work with clinical departments.

The applicant should preferably be under 30 years; salary on scale £4,333 to £7,521 plus £740 London Weighting Allowance. Further details from Professor Brenda E. Ryman, Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF.

Applications on forms obtainable from the School Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St. Dunstan's Road, London, W6 8RP. (Ref: 078) 3634(A)

UNIVERSITÄT FREIBURG
LEHRSTUHL FÜR ZELLBIOLOGIE
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for this post to work on rRNA-binding proteins of the nuclear matrix. Experience in fractionation techniques of RNA and/or protein would be advantageous. The post will be for two years with possibility of renewal. The salary will be according to BAT II a (about 42,000 to 45,000 DM p.a.)

Applicants should write, enclosing full curriculum vitae, to: Dr F. Wunderlich, Lehrstuhl für Zellbiologie, Institut für Biologie II, Universität Freiburg, Schänzlestr. 1, 7800 Freiburg, Germany-W. Tel.: 761-2032680 W486(A)

UNIVERSITY OF PENNSYLVANIA
THE MOORE SCHOOL OF ELECTRICAL ENGINEERING
POSTDOCTORAL POSITION

The Moore School of Electrical Engineering, University of Pennsylvania invites applicants for a postdoctoral fellowship in chemical sensing at elevated temperatures. The research will involve the investigation of semiconductor devices in high temperature operation. A background in one of the following areas is required: electrical engineering, physics or chemistry. An interest in electronics and a working knowledge of computers is desirable.

Send resume and two letters of reference to Prof. J.N. Zemel, The Moore School of Electrical Engineering, D2, Department of Electrical Engineering and Science, University of Pennsylvania, Philadelphia, 19104. An equal opportunity, affirmative action employer. W481(A)

BLOOD PRODUCTS LABORATORY VACANCIES

Applications are invited for two positions in the Research and Development Department to work on new methods of plasma protein fractionation.

Protein chemistry, chromatography, electrophoresis and ultrafiltration are important features of the work together with the assessment of new plant and equipment in relation to the development of new procedures within a production environment.

Scientific Officer (REF. BPL/2)

The successful applicant is expected to possess Phd level qualifications in Biochemistry or a related life science and preferably experience applicable to the stated research problems.

Remuneration will be commensurate with qualifications and experience on a salary scale of £4,261 to £6,627 (under review) plus London Weighting. NHS superannuation scheme applies.

Technician (REF. BPL/18)

Applicants should possess a BSc, or its equivalent, in Life Sciences (preferably Biochemistry) and have experience in chromatographic and electrophoretic techniques. Salary is expected to be in the range of £3,768 to £5,391, plus outer London weighting. NHS superannuation scheme applies.

Application forms, available from Mr G.M. Bailey, Blood Products Laboratory, Dagger Lane, Elstree, Herts, WD6 3BX, should be submitted within 28 days quoting the appropriate reference number.

3636(A)

SCIENTIST BLOOD PRODUCTS

We require a scientist with a PhD in chemistry, biochemistry or the biological sciences, with some post doctoral experience, to work in the Department of Blood Products. Knowledge and interest in haemostasis is highly desirable. The person appointed will be responsible for quality control tests on certain blood products and will also have opportunities for individual and collaborative research, both locally and internationally.

The appointment is for 5 years in the first instance. Salary is on MRC scales, depending on age and experience.

Please write or telephone for further details and an application form quoting reference BL/035 to the Personnel Officer, The National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232 ext. 200.

NIBSC

National Institute for Biological Standards and Control

3635(A)

UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF PHARMACY

INHALATION AEROSOL RESEARCH

A programme designed to optimise inhalation aerosols as a systemic drug delivery system will evaluate the effects of: 1) Formulation factors; 2) Physiological variables, and 3) Methods of aerosol generation upon the bioavailability of a marker drug administered in inhalation aerosols to experimental animals.

Applications are invited from persons with suitable post-graduate qualifications for the post of Research Fellow or Officer. Starting salary will be in the range £4333 to £4910 per annum. The programme will continue for a 3 year period but the appointment will be renewable on an annual basis.

Informal enquiries may be directed to Dr Peter R Byron, Tel 021 359 3611 Ext 277.

Application forms and further particulars may be obtained from the Staff Officer (quoting Ref No 381/6) University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. (Tel: 021 359 3611 Ext 201) and application should be returned not later than 23rd November 1979. 3625(A)

University of Bradford

SCHOOL OF STUDIES IN SCIENCE & SOCIETY

Applications are invited for the post of

RESEARCH ASSISTANT

to work on a project concerned with the metabolism of 2.4D in plants.

Applicants should possess a good honours degree and have experience in the isolation and identification of plant hormones, and intermediate products. The appointment will be for one year.

Salary within range: £3,775 to £5,488 p.a. Further particulars/application forms obtainable from the Registrar, Ref: SS/RA/N University of Bradford, West Yorkshire, BD7 1DP. Informal enquiries to Professor Tom Stonier, School of Science & Society. 3640(A)

MEDICAL RESEARCH COUNCIL

DUNN CLINICAL NUTRITION CENTRE

Cambridge

NUTRITIONIST

Applications are invited for a Nutritionist with an interest in Medical Epidemiology to participate in a two year programme funded by the EEC Biology Medical Research Division concerned with food intake data in EEC countries. In addition to the collection and examination of existing data the applicant would be expected to work with a medical epidemiologist in the planning of future work to collect further data and to test various hypotheses on the role of dietary factors in the aetiology of common diseases.

Knowledge of French and/or German would be an advantage and a readiness to travel is essential. Experience in dietary assessment techniques and in the handling of large quantities of data will be required.

Salary according to experience.

Further details from and application to Dr W P T James, Assistant Director, Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Trumpington Street, Cambridge CB2 1QE. 3643(A)

The Department of Pathology at the University of Pittsburgh School of Medicine wishes to recruit a member for its Division of Experimental Pathology. The position will entail research and teaching to medical students and to graduate students. There are ample clinical opportunities for research programs needing them. The major areas of interest in the Division are immunology and tumor biology.

The level of academic appointment will be commensurate with the candidate's qualifications.

Reply with Curriculum vitae to: Thomas J. Gill, MD, University of Pittsburgh School of Medicine, Department of Pathology, Room 716, Scaife Hall, Pittsburgh, PA 15261.

An equal opportunity/affirmative action employer. W495(A)

THE UNIVERSITY OF TEXAS AT AUSTIN

ASTRONOMY DEPARTMENT

invites applications for

TWO ASSISTANT PROFESSOR POSITIONS

These positions are regular, tenure-track assistant professorships with duties that include both teaching and research. Available research facilities include: the optical telescopes (2.7, 2.1, 0.9, and 0.8 m) of McDonald Observatory, the 2 mile 82 cm interferometer of the University of Texas Radio Astronomy Observatory, the 4.9-m antenna of the Millimeter Wave Observatory, as well as the CDC Cyber 170/750 campus computers.

Applications should include: a description of educational background, teaching, and research experience; a list of all publications; preprints of papers submitted but not yet published; and a statement of current research interests and future plans. Applicants should request at least three scientists who know the applicant personally to send letters of recommendation directly to: P. Vanden Bout, Chairman, Astronomy Department, RLM 15.220, University of Texas at Austin, Austin, TX 78712. Applications and letters of recommendation should be received by 1980 January 15.

The University of Texas at Austin is an Equal Opportunity Employer with an Affirmative Action Program. We encourage and welcome applications from all qualified persons regardless of sex, race, creed, national origin, or non-disqualifying handicap. W480(A)

A VACANCY EXISTS in the Department of Biochemical Pharmacology, Institute of Cancer Research (Sutton) for a post-doctoral fellow to join a small group of biochemical pharmacologists investigating the metabolism and modes of action of new and established anticancer agents. At present the major interest is in the field of antimetabolites. Candidates with broad interests in biochemistry and pharmacology are invited to apply. Salary will be in the range £4,910 to £7,521 pa (under review) plus London Allowance of £740 p.a.

Handwritten letters accompanied by a detailed curriculum vitae (in duplicate) and with the names of two referees should be addressed to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref 300/G/19. 3606(A)

UNIVERSITY OF HONG KONG

LECTURESHIP IN MICROBIOLOGY

Applications are invited for a post of Lecturer in the Department of Microbiology. Applicants who are not medically qualified will also be considered.

Annual salaries (superannuable) are: Clinical Lecturer HK$68,580 — 72,060 — 83,340 — 87,240 — 91,320 BAR 109,920 — 114,840 — 119,760 — 124,680 — 129,600.

Non-clinical Lecturer HK$67,920 × 4,560 — 77,040 BAR 81,600 × 4,560 — 113,520 (£1 = HK$10.70 approx.) Starting salary will depend on qualifications and experience.

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Recruitment Section, Secretary's Office, University of Hong Kong, Hong Kong. The closing date for applications is 30 November, 1979. 3603(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts AL5 2JQ

Temporary SCIENTIFIC OFFICER

for one year from 1.12.79 to assist a team working on the genetic control of storage protein synthesis in barley and other cereals. The post is funded by the EEC.

Qualifications: BSc degree or equivalent qualification in a Biological subject with previous experience in Biochemistry. Salary £3,289 or £3,470 (£3,591 or £3,809 pa from 1.1.80), according to qualifications and experience.

Apply in writing to the Secretary giving names and addresses of two referees, and quoting Ref. 414 by 8th November, 1979. 3624(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY PATHOLOGY

Applications are invited for the post of Lecturer in the Department of Veterinary Pathology.

Preference will be given to veterinary graduates with interests and training in Clinical Chemistry, but applications are also invited from biochemists or recent veterinary graduates contemplating a career in this field.

The salary scale is £4,333 to £8,992 per annum.

Applications, together with the names of three referees, should be received not later than 15 December 1979 by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref: RV/825. 3571(A)

UNIVERSITY OF DUNDEE

Applications are invited for a newly established

CHAIR OF ZOOLOGY

in the Department of Biological Sciences. There is at present one other chair in the Department, the Boyd-Baxter Chair in Biology which is currently held by Professor W D P Stewart, FRS.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN, with whom applications (ten copies or, if posted overseas one copy) should be lodged by November 22nd, 1979. Please quote reference EST/86/79J. 3576(A)

UNIVERSITY OF OXFORD

Genetics Laboratory

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT GRADE 1A Ref: G/R/8

Applications are invited for the position of Post Doctoral Assistant (Grade 1A) funded by a grant from the Medical Research Council awarded to Dr I W Craig. The research concerns the characterisation of monoamine oxidase expressed in various human tissues and by cells in culture, and the application of somatic cell genetic analysis to the identification of genes responsible for the expression of this enzyme. Experience in the techniques of radioimmuno assay and of translation *in vitro* is desirable. The appointment, starting as soon as possible after 1st March, 1980, is likely to be for two years.

Salary on the scale £4333 — £6066.

Application, as soon as possible, including the names and addresses of 2 referees to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. 3649(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF METALLURGY & MATERIALS SCIENCE

Pembroke St. Cambridge CB2 3QZ

POSTDOCTORAL RESEARCH ASSISTANT

The successful applicant will have expertise in transmission electron microscopy and a PhD in Metallurgy & Materials Science or in Physics. The project is to study (using electron microscopy; techniques to include hot-stage, X-ray analysis, high resolution and high voltage) the nucleation, microstructures and growth kinetics of intermetallic compounds in the reaction layers which grow during the fabrication and/or heat-treatment of composite A15 superconductors. There will be close collaboration, in an interactive programme, with an existing group investigating and optimising superconducting properties and with an industrial scale fabrication facility at Harwell. Prior research experience involving superconductors is not required.

Salary is on S.R.C. scales and the appointment is for three years commencing from 1st October, 1979 or as soon as possible thereafter.

Applications, together with curriculum vitae, should be sent to Dr J.E. Evetts or Dr E.R. Wallach at the above address. 3551(A)

UNIVERSITY OF EDINBURGH

RESEARCH ASSOCIATE IN CARDIOLOGY

A Research Associate in Cardiology is required to join an investigation team undertaking experimental studies into basic aspects of coronary heart disease. A good honours degree in Physiology or Pharmacology would be an advantage. Opportunity for study for a higher degree.

Initial salary within the range £4,333 to £5,488 p.a.

Applications to Professor M.F. Oliver, Department of Cardiology, Royal Infirmary, Edinburgh, by 30th November 1979. Please quote Reference 5062. 3639(A)

UNIVERSITY OF OREGON HEALTH SCIENCES CENTER

DEPARTMENT OF NEUROLOGY

POSTDOCTORAL RESEARCH POSITION

Position available immediately in physical or biological chemistry for research on cell surface chemistry and cell separations based on the membrane properties in two phase aqueous polymer systems.

Salary: $15,000 to start with increments in subsequent years.

Apply with résumé to Dr G.V.F. Seaman, Department of Neurology, University of Oregon Health Sciences Center, Portland, Oregon 97201. W488(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

TECHNICIAN 3 (Biochemistry)

equired to run a tissue culture facility. Qualification — O.N.D. or equivalent. Experience in tissue culture not required as raining will be given. The appointment is or two years and is sponsored by the Lord Dowding Trust Fund. Emoluments in the ange £3122 — £3553 plus £325 London Weighting Allowance. Application forms nd further particulars from the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London, SW17 ORE. Closing ate 22 November 1979. 3653(A)

POST DOCTORAL POSITION

One Biochemist

One Neuroendocrinologist (with either a neurophysiological or biochemical background)

For studies on the developmental endocrinology of the pig foetus. Both positions tenable for an initial period of 12 months, starting 1980.

Applications including a curriculum vitae, full details of qualifications and the names of two referees should be sent by November 15, 1979 to Forschungsbereich Endokrinologie und Neuroendokrinologie, Institut für Tierzucht und Tierverhalten FAL, Mariensee, 3057 Neustadt 1, Federal Republic of Germany. W490(A)

HARVARD UNIVERSITY

BIOLOGICAL OCEANOGRAPHER

Harvard University seeks to appoint a tenured professor in the field of Biological Oceanography. Preference will be given to oceanographers with (1) a strong field and laboraory program dealing with the pelagic realm and encompassing work on the interaction between organisms and their environment or on groups of organisms, possibly including emphasis on a specific group; and (2) ability to offer high quality instruction in biological oceanography at both graduate and undergraduate levels.

Applicants should send a curriculum vitae and statement of research interests to: Farish A. Jenkins, Jr., Chairman, Department of Biology, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138.

Harvard is an Equal Opportunity/ Affirmative Action Employer. W493(A)

CHURCHILL HOSPITAL RESEARCH INSTITUTE (University of Oxford)

Applications are invited for a BIOLOGIST

to join a research team investigating the effects of therapeutic doses of x-rays and fast neutrons on normal tissues. The successful candidate will be expected to participate in studies aimed at examining both physiological and histological changes in tissues after irradiation. The post, which is grant aided, is suitable for someone with a higher degree or equivalent research experience.

Further information and applications, with the names of two referees by 16 November, to Dr J.W. Hopewell, Churchill Hospital Research Institute, Headington, Oxford. 3549(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATE IN THEORETICAL PHYSICS

Applications are invited for a Science Research Council funded Research Associate to undertake a theoretical investigation of electron energy loss mechanisms in solids under the supervision of Professor J. L. Beeby. The Research Associate must have at least three years' postgraduate research experience in some aspect of theoretical physics, preferably solid state physics. The appointment will be for three years with a starting salary not less than £4,333.

Applications should be sent to Professor J.L. Beeby, Department of Physics, The University, Leicester LE1 7RH. 3610(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF GEOLOGY

Prior to application, further information (including application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, ext. 431.

The closing date for receipt of completed applications is Thursday, 6th December, 1979. 3623(A)

EAST MALLING RESEARCH STATION

SUPERVISOR OF STATISTICAL COMPUTING SERVICES

Supervisor of data preparation and programming for the analysis of results of horticultural experiments on the Agricultural Research Council's dual ICL system 4 based network, with local RJE backed by a PDP8A mini-computer at internationally renowned Research Station conducting a wide range of basic and applied research.

Applicants must have degree in computing science with mathematics or statistics as a subsidiary or HNC in Computing science or equivalent and at least 5 years post qualifying experience for appointment at the higher grade.

Salary in scale £3,289 to £5,085 (£3,591 to £5,486 from 1.1.80) or £4,727 to £6,211 (£5,097 to £6,737 from 1.1.80) depending upon qualifications and experience. Non-contributory pension scheme.

Further details and application form from The Secretary, East Malling Research Station, Maidstone, Kent. 3599(A)

THE UNIVERSITY OF BRITISH COLUMBIA CLINICAL ENGINEERING PROGRAM

Applications are invited for the position of Director of Engineering Program. The director's responsibility will be the operation and development of a master-level program approved to start in September 1980. The director must hold an advanced engineering degree (PH.D. preferred), have several years clinical experience and be eligible for registration as a professional engineer in British Columbia. The appointment will be at the Associate Professor Level and applications should be sent, by January 1980 to: Professor Axel Meisen, Chairman Clinical Engineering Search Committee, Faculty of Applied Science, University of British Columbia, Vancouver, BC V6T 1W5. Canada. (Late applications may be considered). W503(A)

SCUOLA NORMALE SUPERIORE Pisa, Italy

POST-DOCTORAL POSITION

in Theoretical Physics is offered for a period of 10 months from 1 October 1980, for research in one of the fields: *astrophysics, plasma physics, particle physics, and field theory.*

Applicants may be of any nationality but must not be over thirty five on January 15, 1980.

The total salary attached to this position is 7 milion Italian lire. Successful candidates may apply for a partial reimbursement of travel expenses.

Applications should be sent by January 15, 1980 to the *Director of the Scuola Normale Superiore, Piazza dei Cavalieri, 56100 Pisa, Italy,* and should enclose a curriculum vitae, a detailed programme of study, and letters from three referees. W482(A)

UNIVERSITY OF DUNDEE

DEPARTMENT OF PHARMACOLOGY & THERAPEUTICS

Applications are invited for a new

CHAIR OF PHARMACOLOGY

to be filled within the Department of Pharmacology and Therapeutics from 1 October 1980. This will be a second Chair within the Department. The other Chair is that of Therapeutics held by Professor J. Crooks who, at present, serves as Head of the Department.

The salary will be at an appropriate point within the professorial range for UK universities.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN, with whom applications (twelve copies) should be lodged by 29/11/79. Please quote reference EST/78/79J. 3626(A)

UNIVERSITY OF CAPE TOWN

DEAN OF THE FACULTY OF SCIENCE

The post of permanent full-time Dean of the Faculty of Science at the University of Cape Town will become vacant by the end of 1980 on the retirement of the present incumbent, Professor J S de Wet.

It is hoped that the new Dean will assume duty early in 1981, but the timing is flexible. We are seeking a scientist of distinction and standing who will be able to provide the necessary academic leadership. Interested persons should in the first instance contact Professor C von Holt, Chairman, Search Committee, Department of Biochemistry, University of Cape Town, Private Bag, Rondebosch 7700, South Africa. Alternatively, we would welcome suggestions for suitable candidates from our colleagues in the academic community. W501(A)

APPOINTMENTS VACANT

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ZOOLOGY
A RESEARCH ASSISTANT

A Research Assistant with an honours degree in Zoology, Marine Biology or Biology is required to work with Dr D M Holdich (from 1 January, 1980) in the above Department for 3 years on a NERC-sponsored project on the systematics and ecology of British marine tanaid crustaceans. The work will involve systematic analysis of collections of material coupled with ecological observations on shallow water species off the west coast of Scotland. An aptitude for micro-anatomical work would be an advantage. Applicants should hold a current driving licence.

Salary commencing at £3775 per annum.

Registration for a higher degree may possibly be allowed.

Further details can be obtained from Dr D M Holdich. Applicants should send a curriculum vitae and names of two referees as soon as possible to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. 3654(A)

UNIVERSITY OF READING
PLANT SCIENCE LABORATORIES
POSTDOCTORAL RESEARCH FELLOW

required in Department of Botany to continue an investigation, funded by Cancer Research Campaign, into the metabolism of nucleic acids during meiosis and gametogenesis in eukaryotic organisms. Preference will be given to applicants with a strong cell-physiological background, especially those with experience in electron microscopy.

The post is initially for a fixed period until 30 September 1980. It is hoped that there will be an extension until 30 September 1981 but this cannot be guaranteed.

Salary in range £4,333 to £5,199 p.a. plus USS superannuation. Apply, quoting Ref.MN78A, with curriculum vitae and names of two referees to Assistant Bursar (Pesonnel), University of Reading, Whiteknights, Reading RG6 2AH. 3621(A)

UNIVERSITY OF PITTSBURGH
DISTINGUISHED PROFESSORSHIP

A Chair of Molecular Biology is to be established within the Department of Biological Sciences. Conditions of employment, i.e. salary, lab space, funds for equipment and remodeling, teaching load, are exceptionally attractive.

Consideration will be given to truly outstanding investigators regardless of present rank working in diverse fields (i.e. neural and endocrine function, cell biology, gene regulation, etc.).

Curriculum vitae should be sent by individuals applying directly or by nominators to: Maurice Sussman, Professor and Chairman, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

The University of Pittsburgh, acting in the spirit and the letter of Affirmative Action regulations, actively encourages applications from women, minority members, and other individuals covered by the mandates of Title IX. W492(A)

FELLOWSHIPS STUDENTSHIPS

POSTDOCTORAL RESEARCH ASSOCIATESHIPS AT THE NAVAL MEDICAL RESEARCH INSTITUTE BETHESDA, MARYLAND

Sponsored by
The Naval Medical Research and Development Command
in association with
The National Research Council

Competitive applications are now being accepted for postdoctoral research associateships, starting 1 October 1980, in the areas of behavioral sciences, environmental stress, immunology, microbiology, parasitology, pathology, toxicology, and underwater biomedical research. Candidates must have received their doctoral degree (MD, PhD, DVM, or ScD) within the last five years. Salary will be $19,263 per annum.

Completed application forms are due not later than 15 January 1980. Information and application forms may be obtained by writing to the National Research Council Associateship Office (JH 608-N3), 2102 Constitution Avenue, NW, Washington, DC, 20418. An Equal Opportunity Employer. W497(E)

UNIVERSITY OF WESTERN AUSTRALIA
Perth
THE RAINE RESEARCH CENTRE FOR PERINATAL AND DEVELOPMENTAL BIOLOGY POST-DOCTORAL RESEARCH FELLOWSHIPS

The Centre is seeking doctoral graduates with experience in the following areas of research: Developmental cell biology, eg erythropoiesis or myogenesis; Molecular biology as applied to cell differentiation; Membrane biology and/or intracellular organelle synthesis; Electron microscopy applied to reproductive and/or developmental biology.

The Centre is situated in the Departments of Anatomy and Human Biology, Biochemistry and Physiology of the University of Western Australia. The Fellowships are intended for PhD graduates or those with equivalent qualifications, who by publication and in other ways have demonstrated significant research capability. The Fellowships will commence in 1980. Appointments will be made for two years in the first instance with the possibility of renewal for up to a further three years.

Commencing salary: $A16,291 per annum. The appointees will be entitled to an allowance of up to $A1,975 towards appointment expenses.

Further information about the Centre and its research activities and facilities may be obtained from Professor E.H. Morgan, Department of Physiology.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Acting Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009 by 15 December, 1979. Candidates should request three referees to write immediately to the Acting Staffing Officer. 3628(E)

Postdoctoral Fellowship

Applications are invited for a NRSA postdoctoral fellowship in association with a multidisciplinary Hematology Training Program at the Sloan-Kettering Institute for Cancer Research. The primary goals of the program are to train and conduct research in the following areas:

1. **Identification and characterization of erythropoietin responsive cells**
2. **Refinement of clinical bone marrow transplantation**
3. **Genetic disorders in red cell enzymes**
4. **Interaction of leukocytes with other hematopoietic elements and**
5. **Fundamental research of HLA**

The awards, for one year with the possibility of renewal, are open to United States citizens or permanent residents who have an MD, PhD or equivalent degree and who have had no more than 3 years postdoctoral experience. Please submit curriculum vitae and at least 3 letters of reference to:

Dr. Malcolm A.S. Moore
Director, Hematology Training Program
Sloan-Kettering Institute for Cancer Research
1250 First Avenue
New York, NY 10021

W496(E)

C.H. BEST FOUNDATION
POSTDOCTORAL FELLOWSHIP

Awarded in molecular endocrinology, molecular biology, differentiation, immunology, structure and function of membranes and lipid metabolism. Tenable for 2 years from 1 July 1980 with a stipend of $15,000 plus travelling and dependent's allowance.

Applicants with less than 2 years of postdoctoral experience should send curriculum vitae and three letters of reference before January 15, 1980 to: Chairman, Search Committee (Dr J. Dorrington), Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6. W484(E)

BRUNEL UNIVERSITY
DEPARTMENT OF BIOCHEMISTRY

Applications are invited from candidates who hold a good honours degree in Biochemistry for research studentships in the department. The studentship stipend and conditions will be those attached to SRC Studentships, and positions are available now.

The areas of study are in cancer research, free radical biochemistry and prostaglandin metabolism.

Applicants should sent full details of their academic career and experience to the Postgraduate Tutor, Dr J.T. Nodes, Biochemistry Dept., Brunel University, Uxbridge, Middx. 3627(F)

The Phyllis and Eileen Gibbs Travelling Fellowship 1980-81

BIOLOGY, ARCHAEOLOGY, SOCIOLOGY AND SOCIAL ANTHROPOLOGY

This Travelling Fellowship has been endowed with the object of assisting women graduates who have considerable experience of research and who intend to undertake fieldwork projects outside the British Isles (and normally not within their own countries of residence) in biology, archaeology, social anthropology or sociology — in that order of preference.

The Travelling Fellowship is open to women who are or have been members of any College or Approved Foundation or Approved Society in, or University Officers in, the University of Cambridge but preference will be given to present or former members of Newnham College.

Further details may be obtained from the College Secretary, Newnham College. *Applications should reach her by 1st January 1980. Please quote N/1/79.*

3631(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF SPACE RESEARCH

RESEARCH FELLOWSHIP

Ionic Reactions of Interstellar, Ionospheric and Environmental Interest

Applications are invited for a United States Air Force sponsored Post-doctoral Research Fellowship within the Ionic Physics Group whose interests are in the study of ionic reactions in planetary atmospheres and the interstellar medium. The successful candidate will be expected to concentrate on some aspects of reactions important in the Earth's atmosphere.

The appointment is tenable immediately until 30th September, 1981. Salary will be on the RF1A scale £4,333 to £7,521, plus superannuation. Maximum starting salary will be £5,199.

Further particulars are available from the Assistant Registrar (Science and Engineering), University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (3 copies) including a full curriculum vitae and naming three referees, should be sent by 16 November 1979.

Please quote ref: NE7. 3612(E)

THE ROYAL SOCIETY

JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1980

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology. This award, which may be held for periods from three months to one year will in 1980 be up to £2,000, adjusted according to qualifications and place and duration of research. Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment. Applications receivable by 15 December 1979 should be made on forms obtainable from The Executive Secretary, The Royal Society, Carlton House Terrace, London SW1Y 5AG. 3605(F)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF PHYSICS

POST-DOCTORAL FELLOWSHIP IN LASER RESEARCH

Applications are invited for the South of Scotland Electricity Board Fellowship in Laser Measurement Techniques, to develop excimer lasers and associated optical systems for studies of atmospheric gases.

The appointment is from 1st February 1980 for about 3 years ending 30th September 1982. Salary will be at the appropriate point on the Research Scale 1A (£4,333 to £7,521 per annum, under review) plus USS.

Applications, with curriculum vitae and names of two referees, should be sent by 23rd November 1979 to the Establishments Officer, The University, College Gate, St. Andrews, Fife, KY16 9AJ from whom further particulars may be obtained. 3618(E)

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE

Department of Applied Biochemistry and Nutrition

Applications are invited for a

CASE STUDENTSHIP

co-sponsored by RHM Research Ltd. The project is concerned with the synthesis and deposition of proteins in developing wheat grains.

Applicants should have at least an Upper Second Class degree in Biochemistry, Applied Biochemistry, Plant Physiology or relevant subjects.

Further details may be obtained from Dr G C Blackwood or Dr G Norton, Department of Applied Biochemistry & Nutrition, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD (Telephone Kegworth 2386) to whom applications giving the names of two referees should be sent as soon as possible. 3648(F)

UNIVERSITY OF CAMBRIDGE

OPPENHEIMER RESEARCH FELLOWSHIPS

Applications invited for Oppenheimer Research Fellowships in the field of colloid science and surface chemistry, including applications to metallurgical reactions. Fellowships tenable for 3 years in a physical or biological science department, commencing 1.10.80, when applicants should be under the age of 30.

Stipend ranges from £4,333 (age 24) to £6,644 (age 32). Contributions towards research expenses and removal from overseas.

Applications to J R Payne Esq., University of Cambridge, 19 Trumpington Street, Cambridge CB2 1QA, by 31.1.80 including curriculum vitae, list of publications, statements of 1,000 words each of current research and proposed programme, names & addresses of 2 persons well acquainted personally and academically with applicant. Decisions will be made by 31.3.80. 3652(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY

RESEARCH FELLOWSHIP

Applications are invited for a three-year MRC-financed research fellowship. Salary will be on the RF1A scale £4333 to £7521, plus superannuation. Starting salary will be up to £5488 according to age and previous (not necessarily related) postdoctoral experience.

The project is to study the chemical changes in virus-infected cells both *in vitro* and *in vivo* by X-ray microanalysis. The work will be carried out in two laboratories under the supervision of Dr J Stephen (Department of Microbiology, Birmingham University) and Dr T.A. Appleton (Department of Physiology, Cambridge University). Full provision has been made for travel between the two institutions and stays of short duration in Cambridge.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (three copies), including full curriculum vitae and naming three referees should be sent by Friday 16th November, 1979. Please quote reference NM6(r). 3573(E)

STRANGEWAYS RESEARCH LABORATORY

BIOCHEMIST OR CELL BIOLOGIST

Applications are invited for a fellowship to study bone formation and the role of vitamin D. The position is tenable for three years with a starting salary, related to the University Lecturer scale, of up to £4,910 depending upon qualifications and experience.

Candidates with some post-doctoral research experience preferred. For further information telephone Dr Ian Dickson (0223) 43231.

Applications with the names of three referees should be sent to: The Administrative Secretary, Strangeways Laboratory, Worts' Causeway, Cambridge CB1 4RN before November 14th. 3615(E)

ASSISTANTSHIPS

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANTSHIP

Applications are invited for a RESEARCH ASSITANTSHIP tenable at either the pre-doctoral or post-doctoral level and supported by the Medical Research Council. The project concerns the role of Ca^{2+} and cyclic-3',5'-AMP in stimulus-response coupling in human blood platelets. Experience and/or an interest in membrane and cyclic nucleotide biochemistry would be an advantage.

The assistantship is tenable from 1st January, 1980 for one year in the first instance with the possibility of renewal for a further two years. Consideration will be given to candidates qualified in disciplines other than biochemistry.

Applicants should send details of their qualifications, together with the names and addresses of two referees to Professor Michael C. Scrutton at the Department of Biochemistry, University of London King's College, The Strand, London, WC2R 2LS quoting reference 191697/N. 3611(P)

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY

ß-Lactam Biosynthesis and Synthesis

POSTDOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited for Postdoctoral Research Assistantships supported by the N.R.D.C. to work on this project. This is a multidisciplinary rsearch effort and posts are available in the following areas:-

Microbiologists/Enzymologists. Experience in handling micro-organisms and cell free preparations would be desirable.

Organic Chemists. Synthetic and biosynthetic experience is required.

These appointments are initially for two years from 1st November, 1979, or as soon as possible thereafter. The posts will be on the Research Support Grade 1A at the appropriate point of the salary scale up to £6,066 p.a., according to age and experience.

Applications, with *curriculum vitae* and names and addresses of two referees, should be sent as soon as possible to Professor J.E. Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. 3617(P)

GRANTS and SCHOLARSHIPS

THE ROYAL SOCIETY

BROWNE RESEARCH FUND
MAURICE HILL RESEARCH FUND
MARSHALL AND ORR BEQUEST

Grants 1980

The Council of the Royal Society invites applications from qualified research workers for personal grants:

(a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

(b) from the Maurice Hill Research Fund (established in memory of the late Dr M N Hill, FRS, for the encouragement of research in physical oceanography and marine geophysics) for research at sea and/or in the laboratory in physical and chemical oceanography, marine geophysics and geology.

(c) from the Marshall and Orr Bequest for the support of marine biological research.

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them. Recipients are permitted to use grants while receiving salaries from regular appointments.

In 1980 the total amounts available to be shared among all successful applicants will be about £4,500 from the Browne Research Fund, £9,500 from the Maurice Hill Research Fund and £7,500 from the Marshall and Orr Bequest; individual grants will not ordinarily be more than £750.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by 15 December 1979. 3607(H)

COURSES

EMBO/FEBS/SKMB Advanced Laboratory Course on "Molecular Biology of Membranes" 14-27 September 1980 Biocenter, Basel Switzerland

The course will include review lectures, specialized lectures and laboratory experiments. Main topics to be covered will include the physical chemistry of membranes, membrane reconstitution and analysis of membranes by biochemical, genetic and immunological methods.

Laboratory space will be available for 24 students. The course fee will include Swiss Francs 100 for registration and approximately Swiss Francs 500 for accommodation and food. Fellowships will be available. Applicants will be selected on a world-wide basis.

Application forms can be obtained from the organizers. Deadline for application is April 1, 1980. Organizers: G. Schatz and J Seelig, Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. Tel. 061/25 38 80. W502(D)

COURSES continued

CARDIOTHORACIC INSTITUTE

British Postgraduate Medical Federation
University of London
Fulham Road, London, SW3
(associated with The National Heart and Chest Hospitals)

COURSE ON PHARMACOLOGY OF ASTHMA

11th — 14th December 1980

This Course is designed for chest clinicians or scientists with an interest in asthma. Lectures, given by hospital and institute staff and by invited speakers, will cover clinical description and management of asthma as well as current views on the proposed mode of action and clinical experience of drug use in this disease.

The fee is £75 which is inclusive of lunches, refreshments and hospitality. There will be a cheese and wine party on Thursday, 13th December at which wives/husbands will be welcome.

Applications should be made to Mr. R. A. Perkins, Secretary, Cardiothoracic Institute, Fulham Road, London, SW3 6HP (Tel: 01-352 8121) from whom a copy of the programme will be available in due course.

3614(D)

London School of Hygiene and Tropical Medicine and The Royal Postgraduate Medical School

(University of London)

MSc IN MEDICAL MICROBIOLOGY

Advanced academic and practical training in bacteriology, virology, immunology, mycology, microbial genetics and medical parasitology as applied to clinical and preventive medicine is provided through an intercollegiate course of study extending over two years.

The first year will be spent at the two Schools of the University followed by a second year at a recognised laboratory. Candidates with satisfactory evidence of prior supervised practical training may be exempted from the second year of study.

The course is open to medical, dental and veterinary practitioners, graduates in science or pharmacy and other suitably qualified students.

Exemption from the Primary Examination for the MRC Path may be granted to successful candidates. The course is accepted as recognised training for College examinations.

A limited number of Medical Research Council awards may be available to suitably qualified British subjects resident in the United Kingdom.

Closing date for applications: 1 March 1980.

Further particulars from the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT. 3597(D)

ANNOUNCEMENTS

A NATO Advanced Study Institute on Chemically Sensitive Electronic Devices will be held at the Peddie School, Hightstown, NJ from 9 June to 21 June 1980. The fundamental principles of transducer action for chemical sensors, the resulting device physics and selected areas of application will be presented in a series of lectures by: P Bergveld and G A Bootsma, Twente Technical University; R Buck, University of North Carolina; G Farrington, General Electric; M Green, Imperial College; T. Matsuo of Tohoku University; I Lundström of Linköping University; I Lauks and J N Zemel, University of Pennsylvania; S Pace, Stauffer Chemical.

This course is primarily intended for the young researcher at the post doctoral level but consideration will be given to anyone in the process of initiating research in these areas. The student body will be sought from a broad range of disciplines such as: electrical engineering, chemistry and chemical engineering, physics, material science and biomedical science and engineering. Limited travel and living assistance is available. Special arrangements are being made to provide low cost travel to and from the Peddie School.

For further information and applications write to either:

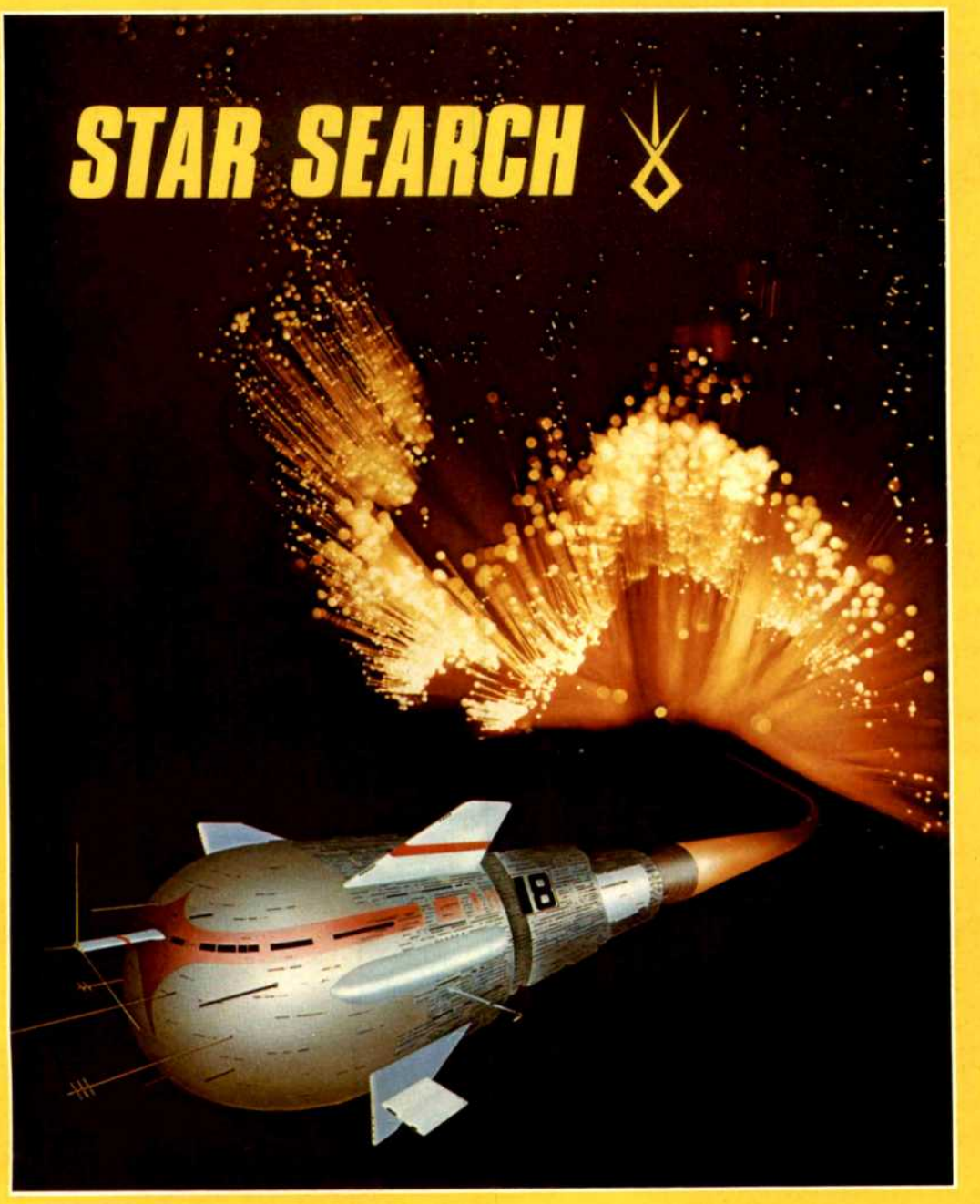
J N Zemel
University of Pennsylvania,
Moore School/D2,
200 S. 33rd Street,
Philadelphia, Pa. 19104. USA.

P Bergveld
Twente University of Tech.
Dept. of Electrical Eng'g.
P.O. Box 217,
Enschede, Netherlands.

W494(G)

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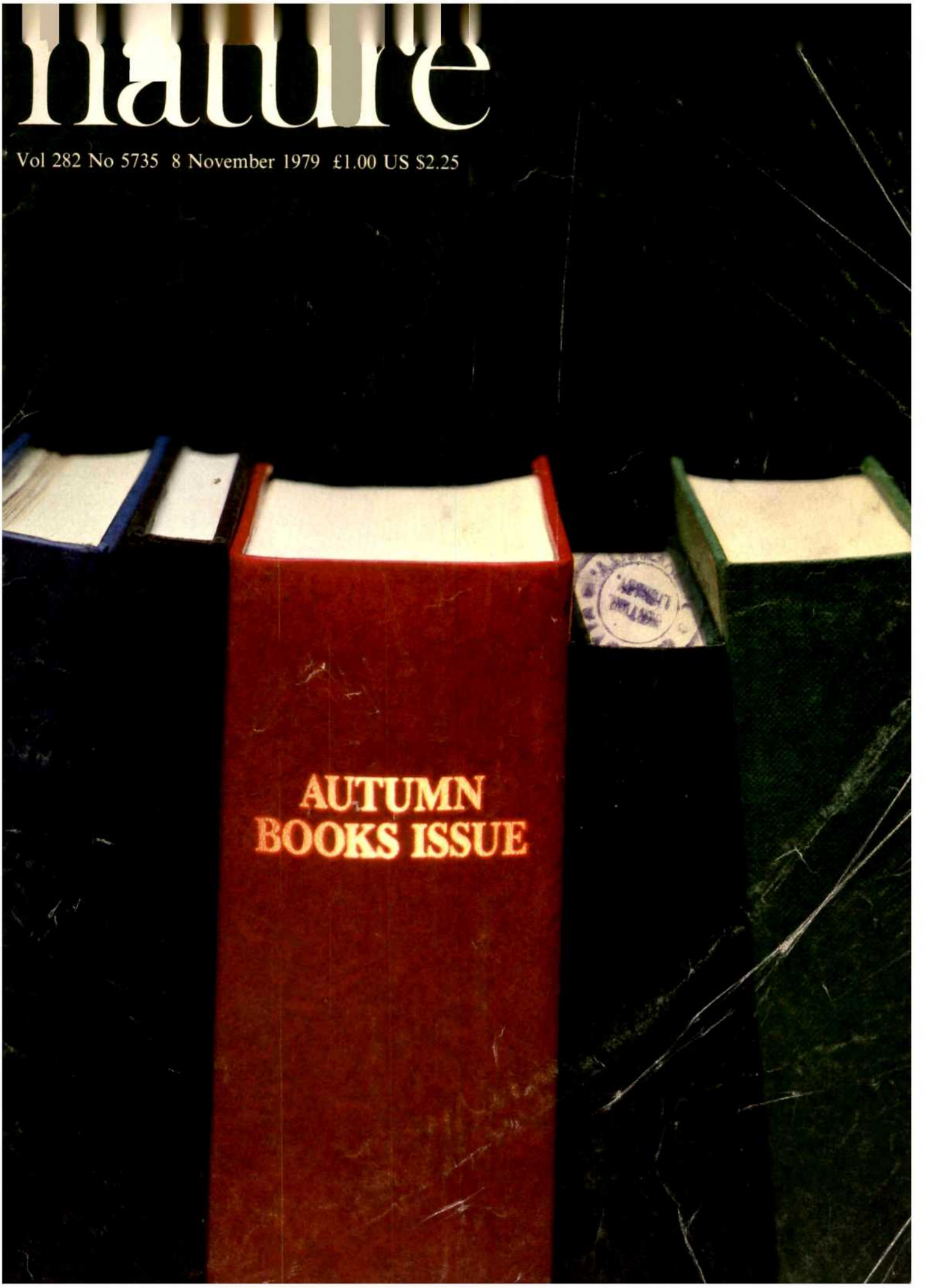
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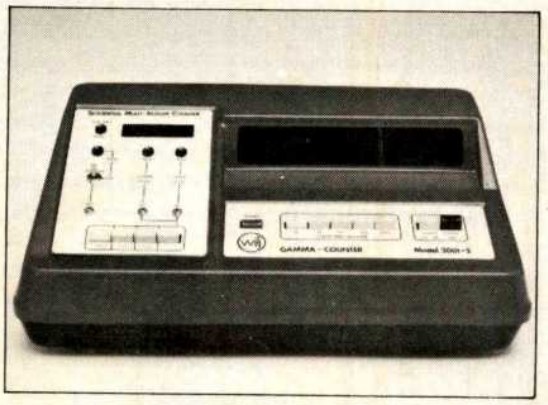
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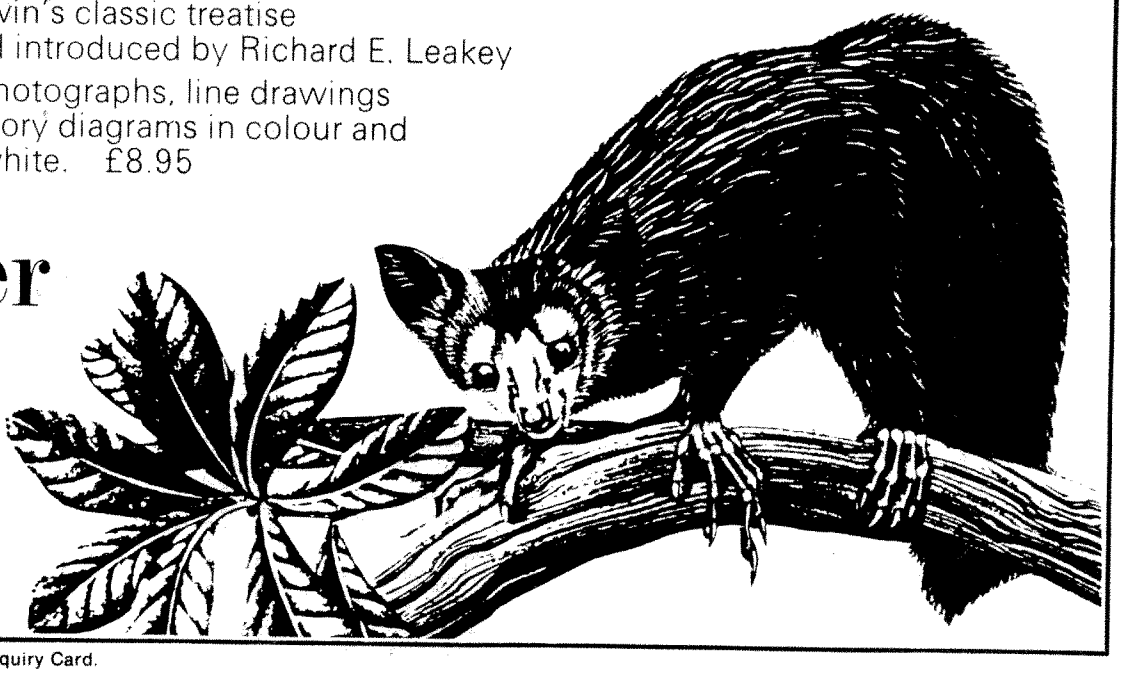
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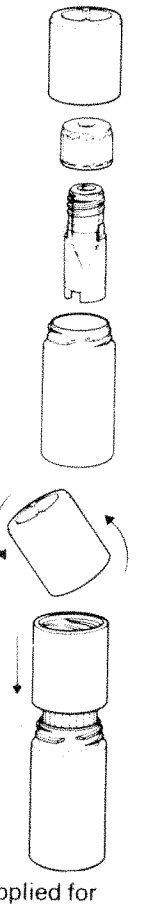
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Neoplastic transformation (Ts'o); Progress with CHO/HGPRT mutagenesis (Hsie et al.); $TK^{+/-} \rightarrow TK^{-/-}$ assay; Mutant analysis (Clive and Moore-Brown); Evaluation of short-term testing (Hollstein); Gene-tox program (Waters)

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1979, 367 pp., illus., appendix
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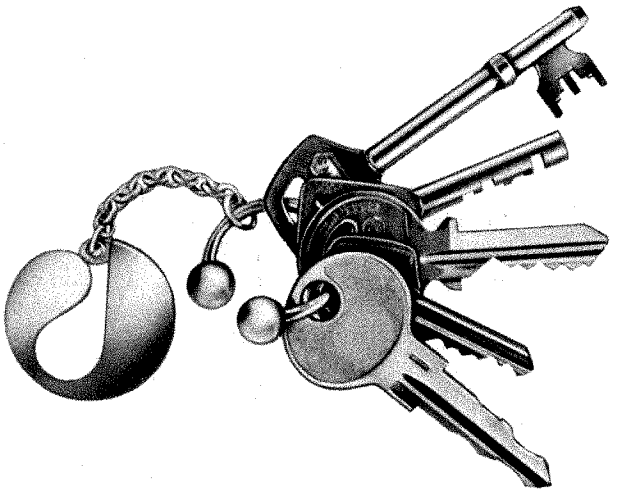
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8 November 1979

Innovation: what's in a word?

There is a certain familiarity to British observers in the set of proposals announced last week to stimulate technological innovation in US industry. "Generic technology centers", based at universities and partly supported by industry, have been an active element of Science Research Council policy for several years. Close university/industry links are already enshrined in the SRC's teaching companies scheme and the now flourishing cooperative awards for science and engineering (CASE) fellowship programme. The National Research Development Corporation will be pleased to learn that the US proposal to establish a network of state and regional corporations for innovation development are partly based on its "successful", example — and there will even be annual "President's awards to industry" similar to those bestowed by the Queen on British industry for over a decade.

In Britain, these various developments have taken place in a piecemeal fashion over a number of years, a gradual process of moulding government intervention in the process of technological development, with increasingly important implications for research policy. In the US, they have been put together into a single package which President Carter referred to as a "first step" in supporting industrial innovation as a spur to international competitiveness and domestic entrepreneurship. And also included in the package are a complex set of legislative and administrative proposals ranging from anti-trust rules to technical information services.

The advantage of such packaging is that it provides a central theme around which otherwise disparate activities can be arranged — and Dr Frank Press, the President's Science Adviser, who initially proposed the studies leading to last week's proposals, points out that one immediate effect has been a heightened awareness of the concept of innovation within the administration. The problem, however, is that innovation itself is a slippery concept that is almost impossible to define. And any claim that it is going down (or even up) can only be made on indirect evidence, such as measurements of productivity, or the level of research and development budgets.

Successful innovation, like invention or creativity, cannot be legislated for. The best that can be done is to identify what are considered to be the key variables, and manipulate them in what is considered an effective way. This is partly a matter of technological and economic analysis. But it can also mean the insertion of political or value judgements necessary to short-cut the analysis and make policy decisions possible.

The danger here is that in the absence of hard data, powerful mythologies may be introduced — and subsequently planned for — to legitimate broad-based policy initiatives. In the UK, one such mythology has been to blame a recalcitrant labour force as the major force obstructing innovation, when in practice the problem is part of a complex of domestic and international processes. Poor labour-management relations may well be a part, but other factors, such as international competition and sky-rocketing energy costs, are just as much to blame.

In the US, there is another myth, that of a lost Yankee ingenuity, the Holy Grail often quoted as the main cause of economic strength and technological superiority, now dimmed by a rising tide of regulation. The problem is that a number of sectors of US industry can be identified — electronics, pharmaceuticals, and biotechnology in general might be quoted as typical examples — where innovation (and profitability) are very far from dead. And again there are external factors, in particular the growing strength of Japan and Germany as technological powers, which may give the appearance of US decline, but can provide only relative rather than absolute measurements.

Certainly the US economy is not as strong as it might be. And declining productivity is an important contributor to double-digit inflation. But there are dangers in reducing the problem to a purely technical one, in particular since this may divert attention from other, equally important, aspects of technological change.

Three of these aspects may be mentioned. The first is the impact of technology on the natural and social environment. Regulation needs to be placed on a rational basis if it is effectively to achieve its intended purpose. In practice, levels of regulation are increasingly being set as the balance of economic against social goals, with the emphasis being shifted back to the former — and this form of rationality speaks less of enlightenment philosophy than of political pragmatism.

The second aspect is the impact of technology policy on developing countries. The United Nations Conference on Science and Technology for Development, held earlier this year in Vienna, drew attention to the ways in which the conditions set by the developed countries for the exploitation of knowledge — for example, on patent and licence policies — can affect the Third World's development prospects. Yet there was virtually no consideration of this particular problem within the US innovation review and the subsequent presidential proposals.

Finally, there are the effects of technological change on the labour force. President Carter, stating that labour's main interest is in early warning about changes, is planning a new labour/technology forecasting system to predict the consequences. But there are many predictions that some aspects of innovation — and in particular the structural unemployment that may well result from the increasing automation of production — will be severe, and that more than advance warning may be necessary.

The Kemeny Commission on the accident at Three Mile Island has warned of the danger of concentrating so hard on the adequate performence of mechanical systems that one fails to pay sufficient attention to the gaps and shortcomings in the social systems which support them. It would be a pity if the current concern with technological innovation were to lead policy-makers further in the same direction. □

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End nuclear complacency — 3 Mile Island Report

"The US Nuclear Regulatory Commission is currently unable to produce an acceptable level of safety for nuclear power" says John Kemeny, Chairman of the President's Commission on the 3 Mile Island Disaster. **David Dickson** reports on the Commission's findings that human failures led to the escalation of what might have been a minor incident into a major disaster



President Carter visits the TMI control room. At the height of the collapse 100 alarms were ringing

THE public inquiry into last March's accident at the Three Mile Island nuclear plant has resulted in a damning indictment of official attitudes towards the safety of nuclear power.

In its final report, presented to President Carter last week, the 12-member commission which carried out the inquiry strongly criticises the nuclear industry for underplaying the risks associated with the operation of nuclear power plants — and the Nuclear Regulatory Commission for failing to apply adequate controls.

The industry has taken comfort from the commission's final decision — agreed despite a number of closely contested votes — not to recommend a moratorium on the construction of new plants until some of the inadequacies which the report identifies have been put right.

But it has not seriously questioned the commission's major finding: that "fundamental changes" are necessary in the organisation, procedures and practices of both the industry and the NRC if similar accidents are to be prevented in the future, and the risks of nuclear power are to be kept within "tolerable limits".

The commission's report is particularly critical of what it calls the "mindset", widely propagated by industry, that nuclear power plants are basically safe. "The Commission is convinced that this attitude must be changed to one that says nuclear power is, by its very nature, potentially dangerous", it states.

The report also points out that the operating company, Metropolitan Edison, failed to change any instructions to its operators following a similar accident which had occurred at another plant in September 1977, despite strong recommendations for making this change by a senior engineer from Babcock and Wilcox.

The report demands wide-reaching changes in the training of both plant operators and managers, expressing concern at the relative neglect of human factors in nuclear safety. The commission says this neglect is itself a product of a "mindset" preoccupied with the safety of equipment, rather than the ability of operators and management to respond to an emergency.

Other recommendations passed to President Carter include proposals that future reactors should be located "in areas remote from concentrations of population", rather than merely using the concept of "low population zones"; that the NRC be reorganised, with a single director replacing the current five commissioners; that an oversight committee on reactor safety be established of up to 15 people from a range of backgrounds to monitor all aspects of the risks of nuclear power; and that a utility applying for an operating licence must show that the state in which its nuclear plant is to be situated has an officially-approved emergency response plan.

How it happened

The report provides a graphic description, based on evidence received over several weeks of public hearings, of how the events unfolded that led to the worst accident in the history of the US nuclear programme. And the description also reveals how human action — and inaction — transformed a series of minor technical failures into a disaster.

According to the commission, the triggering event was a "fairly routine malfunction" in the equipment which purifies the water condensate from the steam turbine inside the plant. The turbine atuomatically stopped, soon followed by the automatic shut-down of the reactor.

To prevent the water pressure inside the reactor vessel from increasing, a pilot-operated relief valve opened automatically; however when the pressure dropped again, the valve failed to close — allowing the coolant water to escape.

Events built up rapidly from there. Confusion over indicator lights led the operators to believe that the valve had closed. A high-pressure injection system came on automatically; but the operators thought this was filling the plant too quickly with water, and cut the flow from 1000 to 100 gallons a minute. The net result of the sequence of events that followed was that the reactor core gradually became uncovered — leading to eventual fears of a core melt-down.

The human failures attract the particular attention of the Kemeny Commission, since without these, it suggests, the accident would have been far less significant. The specific problem was a failure to recognise the actual conditions of the plant; but it relates these to much wider problems of competence, background and training of both operators and management.

"As the evidence accumulated, it became clear that the fundamental problems are people-related problems not equipment problems", the report states. "We do not mean to limit this term to shortcomings of individual human beings — although those do exist. We mean more generally that our investigation has revealed problems with the 'system' that manufacturers, operates and regulates nuclear power plants."

Some of these failures are listed in detail in an analysis prepared by commission staff on the selection, training, qualifaction and licensing of operating personnel. This report points out, for example, that there is no minimum eligibility requirements for operators laid down by the NRC for factors such as education, experience, reliability, or psychological fitness.

Although operator examinations were consistent with regulations, it says, they did not ensure that candidates for a licence had an in-depth knowledge of nuclear reactor theory, design and operation. Training for operators concentrated on systems, equipment and procedures rather than basic scientific and technological concepts, such as thermodynamics, enthalpy, decay heat production, or solid system operation.

(A spokesman for General Public Utilities Corporation responsible for

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running the plant defended their operators' competence by pointing out that over the period 1975 to 1978, TMI control room operators ranked ninth out of 30 facilities in NRC examination performance; commission members say that this merely indicates the size of the problem.)

Another failure is shown up by the short sentence "utility management did not require attention to detail as a way of life at Three Mile Island." The report goes on to list 23 instances, ranging from relatively minor deficiencies in control room practices to major short-comings in failing to correct faulty equipment, or to identify a group with special responsibility for acting on safety concerns raised by employees.

It also raises major questions about the quality control of important components. For example the report points out that although the operating company, Metropolitan Edison, has a quality assurance programme meeting NRC requirements, these did not apply to the plant as a whole, but only to systems classified as 'safety-related'.

The valve which stuck open, was not classified as 'safety-related'. "In addition, the NRC did not require the level of independent review normally found in the quality assurance programmes of safety-critical industries", the report says. Nor did Met Ed go beyond NRC requirements in applying quality controls to equipment such as radiation monitors or control rod drive mechamisms.

It was therefore a combination of poorly prepared operators and management, plus a lack of attention to relatively minor safety details, that made an accident such as that at Three Mile Island "eventually inevitable" says the report. "Large-break accidents require extremely fast reaction, which therefore must be automatically performed by the equipment. Lesser accidents may develop much more slowly and their control may be dependent on the appropriate reactions of human beings", it says.

Basic flaw

Scientists and engineers had worried for decades about the safety of nuclear equipment, but their approach to nuclear safety had a "basic flaw", namely a preoccupation with large-break accidents, and the attitude that if they could be controlled, it was not necessary to worry about the analysis of "less important" accidents.

"This was the tragedy of Three Mile Island, where the equipment failures were significantly less dramatic than those that had been thoroughly analysed, but where the results confused those who managed the accident."

Another problem, says the report, was that information about conditions inside the plant was not presented in the control room in a clear and understandable form. At one point, soon after the valve had stuck open, there were over 100 alarms ringing in the control room, with no system for indicating which way was most important.

Among the other findings of the commission were:

- Health effects: The commission says that the most serious health effect of the accident was severe mental stress, this being highest among those living within 5 miles of the plant, and in families with pre-school children.

Since most of the radiation was contained within the plant — it calculates less than 0.01% of the radioactive iodine released from the fuel escaped into the environment, and that no detectable amounts of caesium and strontium escaped — the health effects of the accident would be minimal. The commission says it can be "practically certain" that there will be less than 10 extra cancer deaths in the 325,000 people living within a 50-mile radius, and that it is highly likely there will be no extra deaths.

- Costs: The commission estimates that, discounting health costs, the cost of the accident will be between $1 billion and $2 billion, much of this being the cost of replacement power. If the plant can not be put back into operation, the cost will be much higher, it says.
- Seriousness of accident: Examination of several different scenarios that could have taken place included some which would have resulted in a "melt-down". Under this scenario, the fuel would reach such a temperature that it would melt through the steel reactor vessel and reach the floor of the containment building, where its heat would be dissipated by water.

The commission calculated that even if a melt-down occurred, there was a high probability that the containment building and the hard rock on which it is built would have been able to prevent the escape of a large amount of radioactivity. But it points out that, since the calculations are at the limits of engineering knowledge, they include a degree of uncertainty.

- Public and worker health and safety: The commission says that there is inadequate knowledge of the effects of low levels of ionising radiation, of strategies to mitigate the health hazards of exposure to radiation, and of other areas relating to regulation setting to protect health and public health.

It points out that state organisation in Pennsylvania did not have adequate resources for dealing with radiation health programmes relating to the operation of Three Mile Island. And that the utility was not required to, and did not, keep a record on workers of the total work-related plus non-work related radiation exposure.

It recommends more emphasis on research on the health effects of radiation co-ordinated under the National Institutes of Health, as well as an increased programme for educating health professional and emergency response personnel in the vicinity of nuclear power plants.

- Nuclear Regulatory Commission: The report spells out in detail criticisms of the shortcomings of the NRC in dealing with the accident, in particular its apparent indecisiveness, and uncertainties in diagnosing the situation inside the plant. It points out, for example, that NRC officials wrongly concluded on the second day that there was a danger that a hydrogen bubble in the reactor vessel might explode through contact with oxygen produced through water radiolysis, which other scientists later pointed out was impossible.

One of the main conclusions of the commission is that the NRC should be restructured. "The totality of our findings is that no-one is running that particular agency."

'Bark worse than bite'

Reaction to the Kemeny Commission's report has been predictably diverse. Nuclear critics argue that the report does not go far enough in following through the logic of its own conclusions, and that the report's bark may therefore turn out worse than its bite.

"We are still left with the problem of the 70 plants that are already operating in this country, many of which should be temporarily shut down until adequate safety can be guaranteed. We need a whole new approach to the safety of our nuclear programme which goes far beyond administrative juggling", says Professor Henry Kendall, professor of physics at the Massachusetts Institute of Technology, and a co-founder of the Union of Concerned Scientists which has been criticising federal safety measures for several years.

The nuclear industry, carefully sidestepping some of the stronger criticism in the report, has interpreted its message as "proceed with caution" — and points out that it has already been doing much to improve safety procedures following the accident.

"It is evident that the commission found nothing in the wake of TMI that suggests that nuclear power plants as presently designed and built are unsafe," says Darl Walske, president of the industry-sponsored Atomic Industry Forum.

But conventional attitudes — the "mindset" referred to by the Kemeny Commission — retain a strong grip. Three weeks ago the *Washington Post* carried a two page advertisement in which Dr Edward Teller claimed "I was the only victim of Three-Mile Island", since he suffered a heart attack resulting from strenuous efforts to answer anti-nuclear critics; the main lesson of the accident, he said, was that "nuclear reactors are even safer than we thought".

Last week NRC commissioner Peter Bradford wrote to the newspaper accusing Dr Teller of misleading the public — and pointing out that the advertisement had been paid for by the firm which produced the valve which had stuck open at Three Mile Island. □

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US boosts university-industry links

After 18 months' study by the Commerce Department, President Carter last week made 32 proposals for increasing the commercial exploitation of scientific knowledge. **David Dickson** reports.

PRESIDENT Carter is demanding that federal agencies substantially increase their efforts to stimulate links between the university research community and private industry. Building on the recent experience of the National Science Foundation, he has recommended that the government raise its support for such efforts by $150 million a year.

The recommendation is one of 32 which the President made last week in a message to Congress on new initiatives to stimulate industrial innovation.

Describing the package of recommendations, the fruits of an 18-month study by the Commerce Department, as a "first step" towards meeting the nation's commitment to innovation, he said they would help "the continuing challenge to maintain the economic strength of the US economy."

The review was initially recommended by Dr Frank Press, director of the Office of Science and Technology Policy. Its basic premise was that many of the nation's economic problems can be related to a decline in the rate of productivity; and that this in turn has been partly caused by a decline in innovation, as implied by factors such as declining investment in research and development and a decrease in the number of patent applications.

Many of the President's recommendations directly involve research. Yet rather than concentrate on ways of increasing direct federal support for research of interest to industry, the President's recommendations focus instead on the environment in which both research and innovation take place — and thus how the government can alter this environment to make it more conducive to economic growth.

The review therefore covered all aspects of the innovation process, from anti-trust policy to technical information systems, making numerous proposals for legislative and administrative changes. In the short-term, however, a principal beneficiary of the review is likely to be the National Science Foundation, budgeted to receive about $30 million of the extra $55 million which the President suggested needs to be spent in 1981.

The purpose of the NSF money will not primarily be to support further research — although this will obviously be one effect — but to open up the arteries connecting universities to private corporations. In this way it is intended to help to lead to what Secretary of Commerce Mrs Juanita Kreps referred to as "a more effective partnership among government, industry, labour, and the academic community."

In particular, the NSF's budget request for 1981 will include a three-fold increase in support of a programme introduced last year to finance research proposals jointly submitted by university and industry research workers. NSF officials points out that the programme, scheduled to increase from $8 million to almost $30 million, has already attracted the involvement of major corporations such as Westinghouse, Union Carbide and Monsanto.

President Carter has now suggested that the NSF experience be used as a test-bed for cooperative projects sponsored by other federal agencies, such as the Department of Defense, the Department of Energy and the Environmental Protection Agency, and that an aggregate target of $150m be eventually aimed for.

Furthermore in view of the historical importance which small entrepreneurial firms have played in bringing new products to the market-place, he is proposing to increase the budget of the NSF's Small Business Innovation Research Programme from $2.5 million to $10 million. Again this would be used as the basis of a $150 cross-agency effort.

"The innovation review makes clear

The new innovation initiatives in detail

SOME of the main initiatives announced by President Carter to spur industrial innovation in last week's message to congress:

●Four "generic technology" centres will be established at universities and other private sector sites to encourage research and development in fields such as corrosion prevention, automated assembly, and tribology. Each centre will be jointly financed by industry and government, with the latter's share dropping to 20% or less by the fifth year. Three centres will be established in 1981 by the Department of Commerce, and one by the National Science Foundation, at a cost of about $6 to $8 million.

●The NSF will receive an additional $20 million in 1981 to increase its efforts at linking industry with university scientists. The foundation will also work with other agencies to initiate similar cooperative R&D programmes, with an eventual aggregate target of $150 million.

●The federal government will seek legislation to establish a uniform patent policy. Under this legislation, title to a patent resulting from federally-funded research would be retained by the government, but contractors would obtain exclusive licenses in specified fields of use. Small businesses and universities, however, would be allowed to retain patent ownership "in recognition of their special place in our society"

●The Department of Justice will clarify the role of anti-trust legislation with respect to research collaboration between different companies

●The NSF's Small Business Innovation Research programme will be expanded from $2.5 million to $10 million in 1981. The foundation will help other agencies develop similar programmes, under the co-ordination of the Office of Management and Budget, with an aggregate target of $50 million

●Increased assistance will be provided to establish Corporations for Innovation Development (CIDs) on a state or regional basis, to help entrepreneurs gain access to investment capital

●To help minimise the impact of regulation, executive health, safety and environmental regulatory agencies wil prepare five-year forecasts of their priorities and concerns. "Better knowledge of agency plans will allow industry to plan its research and development" says the administration

●In addition, federal executive agencies responsible for reviewing the safety and efficacy of products will develop and implement a system of priorities. Under these systems, the agencies will identify those products that are most innovative and/or have exceptional social benefits, and expendite their clearance reviews "to the extent permitted by applicable statuttes"

●The Secretary of Labour and the Secretary of Commerce will work with labour and management to develop a national Labour/Technology Forecasting System. This system would develop advance warning of industrial changes and permit timely adjustments

●The Commerce Department and the NSF will host a national conference for deans of business and engineering schools to stimulate improved curriculum development in technology management and entrepreneurship

●The National Technical Information Service will establish a new center, at an annual budget of up to $2 million, whose purpose will be to improve the flow of knowledge from federal labortories and R&D centres to private industry

●The President will establish an annual award for technological innovation in the six areas of transportation, communication, health, agriculture and food, and natural resources (including energy). The awards will be presented by the President's science and technology adviser. □

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how extensively innovation depends on R & D, especially on long-term research. But the expertise in universities has not been used as extensively as possible to support industrial innovation. The NSF programme has already shown successes, and it is time to expand this effort," says Dr Press.

Congressional response to the package, promised since last April, has been lukewarm. Some proposals, such as the setting up of "generic technology centres" and the introduction of uniform patent policy, have been generally welcomed; indeed many are already part of legislative proposals currently before Congress.

At a Senate hearing last week, however, several Congressmen criticised the package for not including any tax incentives related to R & D expenditures. Administration officials replied that these would come later, as part of a complete set of tax reforms; and Dr Jordan Baruch of the Commerce Department said that tax incentives might not be a sharp enough instrument to attack such a complex issue.

Senator Adlai Stevenson criticised the package for lacking imagination. "It is a first step, but I am not convinced that the Executive Branch has got the guts to take the next ones needed to reach a rational technology policy" he said. Mr Stevenson quoted in particular Japan's recently announced plans to stimulate government support across a wide range of technological areas, from ocean mining to integrated circuits.

"For 25 years the question of innovation has been around," replied Dr Press. "For the first time the President has come forward with definite proposals; I think this is an important step. And US industrial leaders that I have spoken to would not want to touch what the Japanese are doing with a ten-foot pole." □

Soviets announce build-up to fusion power

RECENT Soviet developments in energy were conspicuous by their absence from the agenda of last week's UK-Soviet energy symposium in Moscow. To some extext, this was to be expected, since the prime aim of the symposium was to explore areas of possible cooperation in the near future. Nevertheless, even in his exposition of the long-term "second stage" of the new Soviet energy "strategy", the Minister of Power and Electrification, Peter S. Neporozhnyy, made no allusion at all to the Soviet Union's latest step in power generation — the start-up of the first module of the Angara-5 fusion generator.

This is all the more remarkable since the demonstration run of the Angara-5 module, early in October, was given considerable media coverage for both home and foreign consumption. The Angara-5 is an electron beam device, the name, according to *Pravda,* signifying the five tributaries (the beams) which flow into lake Baikal (the tritium/deuterium target), and issue thence as the mighty Angara river (the energy produced). Last month's test of the module was carried out at the Kurchatov Atomic Energy Institute; according to Valerii Legasov, the Deputy Director, after "through experiments" have been carried out with each of the 48 modules, a "demonstration" Angara-5 will be built at the institute's test site.

No indication, has so far been given of when the break even point may be expected, although Moscow radio's "World Service" suggested that the first power plants based on Angara-5 will be built by the end of the century. At all events, practical use of the Angara-5 lies too far in the future to merit a place in Neporozhnyy's "strategy", although his outline of the "second stage" did include some references to MHD and fusion generation.

In MHD, he said, work would go forward on improving conversion efficiency and hinted that "energy and magnetic field parameters close to commercial" were already within reach on one experimental device. Fusion research, he said, would continue using both Tokamak and laser techniques. Already plasma temperatures only 4-5 times less than those required for sustained reaction, he said, had been attained.

The majority of the symposium, however, was devoted to the first stage of the strategy; development of the coal industry, including low grade coals to be used in generating stations close to the mines, expansion of the Siberian hydroelectric network and the phasing out of oil and gas-fired power stations.

Although the Soviet planners still claim ample reserves of oil and gas, they are now ready to admit that these lie in increasingly remote and extreme terrains, and that exploitation costs will become increasingly expensive. Indeed, for the firt time, they are prepared to admit small and temporary stortfalls in production. In the not too far future, the Soviet participants in the symposium agreed, oil will have to be reserved for oil-specific industries — transport and petrochemicals.

Nuclear generation will to a large extent replace oil in the power sector. Although the extension of the Union-wide "supergrid" of 1500 V DC transmission lines should, theoretically, allow power stations to be sited anywhere, the old paradox of the Soviet energy map (80% of resources east of the Urals, 80% of consumption west of the Urals) is being perpetuated in the siting of future nuclear power stations, close to the consumers.

Hardly a mention, even in informal discussions, was made during the symposium of Academician Sollezhal's recent proposals for nuclear generating "complexes" in Siberia. The safety and ecological aspects raised by Dollezhal' were barely alluded to, and the plans for nuclear "boiler houses" for district heating in major cities were given great prominence; with, perhaps, one small difference.

Until now, Soviet commentators have suggested that the nuclear boiler houses would be situated in the suburbs of the cities, with a "safety-zone" of some 1-2 km around them, reserved for industrial and leisure use (but not housing). Now, however, according to Neporozhnyy, the boiler houses may be further away — transmission of the hot water for up to 50km, he said, is reckoned to be "economic". **Vera Rich**

Hungarian reactor to be made 'safe'

CONSTRUCTION of the Paks nuclear power station, which should supply one third of Hungary's present peak power demand, is at last on schedule. Last year, the station, whose construction had been running late since 1975, was the subject of special resolutions by the Council of Ministers and the Party Politburo. A new management system was set up, with government commissioners working on the site. Shortly afterwards, building operations were reported to have "speeded up considerably', although the workforce of 5200 is still insufficient and more managerial changes are envisaged if the original schedule is to be maintained.

This acceleration, however, has not been achieved at the cost of safety. Out of the 60,000 million forints (£1,500 million) which the station will cost, 12,000 million (20%) will be spent on safety measures.

The Hungarian planners are in a somewhat delicate position as regards safety. The country is small, with no convenient expanse of empty land in which to site a reactor. Moreover, Austrian TV reaches considerable areas of Hungary, and although any evidence on the subject can only be anecdotal, there is good reason to believe that the population at large was considerably perturbed by the "anti-nuclear" verdict of the Austrian referendum earlier this year.

Perhaps for this reason, a recent Budapest TV programme describing the Paks station paid especial attention to its safety precautions. "Triple walls", said the commentator, separate radioactive materials from the environment. The outermost of these walls, from his description, is simply a steel and concrete containment vessel — of the type, until recently, dismissed by Soviet planners (who are intimately involved in any Comecon nuclear power station) as a Western capitalist ploy to inflate costs. □

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EPA panel recommends postponement of 2, 4, 5-T hearings

THE herbicide 2, 4, 5-T poses no "substantial hazard to human helath or to the environment" according to a report prepared by the Scientific Advisory Panel of the section of the US Environmental Protection Agency concerned with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Accordingly, the panel recommends that the final hearings to consider the validity of the ban on 2, 4, 5-T be postponed until more information on the carcinogenicity and reproductive toxicity of the herbicide and its 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (dioxin) contaminant is available.

The EPA enforced a ban on the use of 2, 4, 5-T in March of this year following a study in the state of Oregon which linked an increase of spontaneous abortions in the area to spraying with the herbicide (*Nature* 8 March, page 108). The initial ban was restricted to use of the herbicide in forestry but since then has been made total and applies to all applications of 2, 4, 5-T.

Law Court and EPA hearings have been held at intervals since March to consider the merits of the ban. The final hearings, initially scheduled for October 1979 have been postponed until January 1980 and if the advisory panel has its way will be postponed even longer.

The panel's report is based on evidence presented by interested parties at two open meetings held during the summer. Following the meetings, the panel agrees that the EPA is correct in stating that 2, 4, 5-T and dioxin are teratogens and fetotoxins. It also takes the EPA view that as dioxin is carcinogenic in test animals it may be a "potential human carcinogen."

But the panel disagrees with the EPA's opinion that human exposure "from spray drift and the water environment is likely to be broad or substantial" where 2, 4, 5-T use on rice is concerned. As for the use of the herbicide on rangeland the panel says there is no "convincing argument of an immediate or substantial hazard" to humans.

Dow Chemical Company is the major producer of 2, 4, 5-T in the US and stands at the forefront of the campaign to have the ban on 2, 4, 5-T lifted. The company insists that there is no good scientific evidence to support the ban. Dow scientists admit that dioxin is extremely toxic but state that it is not present in sufficient concentrations to pose a human health hazard.

The Advisory Panel points out that on some questions the EPA position is "relatively close to that of the scientists from the Dow Chemical Company". In contrast to the view put forward by the EPA that there is no safe level for dioxin in rats and mice the panel says that such a statement is incorrect. And it suggests that a review of the evidence shows that the EPA and Dow Chemical are not as far apart on this issue as it might seem.

It remains to be seen whether this internal vetting process will have any influence on the EPA's actions on 2, 4, 5-T. The results of the report will give a considerable boost to Dow's campaign and the company for public relations reasons, may well agree with the final recommendations of the panel, that in view of the potential toxicity of dioxin "efforts should be made to further reduce" its level in commerical preparations of 2, 4, 5-T.

Alastair Hay

Pathogens panel should have lay representation, says UK health minister

THE new Conservative government, like its predecessor, is taking into account the recommendations of the Shooter Report on the outbreak of smallpox in Birmingham last year in drawing up new regulations for the safe handling of dangerous pathogens during research work and diagnosis. Mr Patrick Jenkin, Secretary of State for Health, told a meeting organised by the Association of Scientific, Technical and Managerial Staffs (ASTMS) in London last week, that the government is planning to implement many of the Shooter recommendations and also some of those made by the Health and Safety Executive in its recent publication 'Dangerous Pathogens: draft regulations and draft guidance notes'.

Under the government's new proposals, notification of intention to work with category A pathogens (the most dangerous) would be compulsory, said Mr Jenkin, and laboratories would have to be inpsected by HSE inspectors before work could begin. They would then have to be inspected regularly against a checklist which is to be drawn up in accordance with recommendations laid down by Shooter. Laboratories researching with category A pathogens would also have to be sited outside densely populated areas.

The government has set up a panel which is currently looking into the workings of the Dangerous Pathogens Advisory Group (DPAG), a committee of experts currently under the auspices of the Department of Health and Social Security (DHSS) which was set up in 1973 to administer a system of voluntary control of work with category A pathogens. According to Mr Jenkin, the panel is likely to recommend that a new body be set up with an expert and lay membership, including professional, trade union and public interest representatives. This body should remain under the aegis of the DHSS and would administer the new compulsory system of notification.

The position with public health laboratories is different because they are mainly concerned with category B pathogens which do not have to be notified to DPAG. There could come a time, however, said Mr Jenkin, when notification for category B pathogens would also become compulsory. Nevertheless, he assured the meeting that it is still the government's intention to implement within three years the recommendations for improved safety in public health service laboratories laid out in the Howie report published last year. "It would inevitably cost the National Health Service a lot of money to bring all its labs up to the standard of the Howie report", he said, but the government thought that the £7 million (1978 prices) needed could be found.

Clive Jenkins, General Secretary of ASTMS, agreed with the opening up of membership of DPAG to non-experts. "Free standing committees of experts are things of the past", he said and added that a body along the same lines as the Genetic Manipulation Advisory Group (GMAG) would be appropriate: "GMAG has worked as well as is possible for a voluntary body to work". Nevertheless he thought that the Health and Safety Executive would be a much more appropriate body than the DHSS to manage DPAG's affairs. "We want everyone to know the dangers of the leaky labs", he said.



Patrick Jenkin: reforming DPAG

Dr Roger Nourrish, microbiological inspector with the HSE, told the meeting of his visits to research and diagnostic laboratories designed to handle category A pathogens. Of the research laboratories, those at Reading and Paddington had stopped work before the HSE's visit, those at Purbright and Elstree were asked to stop work after the visit until improvements could be made and those at Porton Down and Liverpool were asked to make some inprovements while still remaining operational.

Judy Redfearn

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news in brief

US Supreme Court to rule on whether living organisms can be patented: The US Supreme Court agreed last week to rule on whether patents can be granted on living organisms. The case concerns two patent applications initially rejected by the US Patent Office, but subsequently granted by an appeals court. The patent applications have been filed by research scientists with the companies General Electric and Upjohn respectively; one is for an organism which is capable of breaking down oil spills, the other for a bacteria to produce the antibiotic lincomycin.

The Patent Office, supported by the Department of Justice, contends that living organisms are not covered by current US Patent Law; in support of its claim, it quotes the fact that Congress felt it necessary to pass a separate act to allow the patenting of plants. The Appeals court, however, agreed with the applicants that what was being requested was patent protection for a novel chemical process, and that this comes within the scope of the law as it is now written. In contesting the appeals court ruling to the Supreme Court, the Department of Justice argues that the economic implications of the ruling "are very significant, given the vast area that it opens to patentability."

MIT joins banking firm to study commercial aspects of applied genetics: The Massachusetts Institute of Technology announced last week that it had received a grant of $50,000 from the US Congress' Office of Technology Assessment to co-operate with the New York banking and investment firm F Eberstadt and Co to look at the industrial and commercial aspects of applied genetics. The purpose of the grant, which will be administered through MIT's department of nutrition and food science, is to provide OTA with data for providing Congress with policy options on regulation, taxation, patent protection and research funding in the food, chemical, and pharmaceutical industries that develop and market new products and processes from living cells.

In annoucing the grant, MIT said that after completion of the study, it was planned to hold a seminar for academic and industrial scientists and engineers, and for investors on the development of the industry under study. Five aspects of the industry will be covered in the study: historical development, current and predictable state of the art of various genetic technologies, research efforts in the development and applications of these technologies, and assessment of technical, institutional, economic and social factors that could affect the rate of adoption of these technologies.

French committee formed on recombinant DNA technology: A committee for information on genetic manipulation held its first public meeting 22 October at the Museum of Natural History in Paris. The meeting stressed the need of not acceding to what it called "the giddiness of biologists" and to the "fascination that genetic manipulation exercises on its practitioners". The committee hopes to call attention to the risks inherent in the new technology and to "the great laxness that exists in the definition of health and safety regulations in the area". The Secretary of the group is Jean Deutsch of the Genetic Research Group of the University of Paris VI.

Fewer US physicists means better job propects: The employment outlook of US physicists is improving, according to a study published by the physics manpower panel of the American Physical Society. In an extensive survey of immediate past and future employment prospects for PhD physicists, the panel says supply is beginning to approach demand, and it concludes that "the physics community, taken as a whole, has passed through its gravest hour."

However the panel goes on to warn that the prime reason the job market is beginning to look healthier for new entrants is that there are so few of them. "There will continue to be imbalances between many components of supply and demand", it warns. According to statistics prepared for the panel, the probable number of physics doctoral graduates will coincide with the total demand — assuming a 1 per cent annual growth in the table — around 1984. *"The Transition in Physics Doctoral Employment 1960-1990"*. The American Physical Society, $5.00.

Salam gives Nobel prize money to fund Third World Science: Professor Abdus Salam, co-winner of this year's Nobel Prize in Physics has pledged his share of the prize money — $60,000 — to an international talent fund for young scientists. Speaking at a meeting of the Executive Board of UNESCO, Salam urged increased support for Third World science training because of the failure of the UNCSTD talks last August in Vienna. The developed nations, at Vienna, were asked to contribute $2bn to aid research but had offered only one seventh of that.

Indo-Soviet scientific cooperation to expand: Indo-Soviet cooperation on science and technology will soon cover several new areas. Russia will share with India its experience and technology in earthquake prediction, laser applications, semiconductors and systems analysis over three years beginning 1980, according to a decision taken by the Indo-Soviet subcommission on science and technology which met at Moscow in the second week of October. The areas identified for cooperation in basic sciences are crystallography, low temperature physics, chemistry of natural products, microbiology and radio astronomy. The subcommission will meet again in February 1980 at New Delhi to finalize the objective in each area, time schedule and the participating laboratories.

The two sides have also decided to continue cooperation in alternative sources of energy, MHD generators, metallic corrosion, powder metallurgy, meteorology, low-cost housing, construction technology, scientific and technical information systems, machine tools, and water resource management. Earthquake prediction was included at the insistence of Professor M G K Menon, the leader of the Indian side, since the excellent system developed by the Soviet scientists for predicting and identifying the epicentre of an impending earthquake is of great relevance to the quake-prone Indo-Gangetic plain and eastern India. — *S. Arunachalam.*

Michigan PBB suit settled out of court: The Velsicol Chemical Corporation has made an out-of-court payment of $2.6 million in damages to 70 Michigan farmers who filed suit against the company after buying animal feed which had been contaminated by polybrominated biphenyl (PBB). Millions of farm animals died or had to be slaughtered as a result of the contaminated feed. Velsicol also agreed to waive the farmers' debts to the farm cooperatives that sold the feed, and the farmers agreed to waive any claims of personal injury. According to Velcisol, the latest settlement clears up half of the property claims against the company. There are still 69 suits outstanding.

French government to start Tricastin 1 reactor: The French government has proceeded with the loading of its Tricastin 1 reactor in spite of the presence of cracks in the tubular collar separating the primary and secondary cooling circuits. The decision follows the one taken last week in which the Gravelines 1 reactor was started in spite of similar cracks. Both reactors had been closed because of militant trade union and community response. At this time the Communist trade union federation the CGT has decided not to oppose the loading while the Socialist federation, the CFDT has temporarily postponed further strike action. In the meantime a civil court in Paris has ruled in the government's favour in a civil suit brought by Friends of the Earth. The court ruled that it was incompetent to decide whether to grant a request by Friends of the Earth to halt the start up until the cracks are repaired or the plates are replaced.

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The Asian Institute of Technology celebrates its 20th anniversary this year. David Bousfield and Ziauddin Sardar have visited it recently and have returned with different views on the impact it is making on the regional development of Asia. Here they discuss their impressions.

Could AIT be a model for the Third World . . .

THE role science and technology should play in developing countries may well be epitomised by the approach adopted by the Asian Institute of Technology (AIT) situated in Thailand about 40 Km north of Bangkok.

More than half the world's population live in the countries served by the AIT and for the past twenty years it has been gradually building up scientific and technological knowhow in the developing nations of the East. The recent UNCSTD conferences left many scientists confused as to the role they could and should play in aiding development in the Third World — the example set by AIT may help them and their governments to see a way to help.

The Asian Institute of Technology started life in 1959 as the South East Asian Treaty Organisation (SEATO) Graduate School of Engineering — the brain child of Pote Sarasin, the first Secretary General of SEATO, and a former Prime Minister of Thailand. Intended to provide a source of highly qualified engineers for Asian projects, the school began as a small collection of rooms in the Engineering Faculty of Chulalongkorn University deep in the heart of Bangkok. But it grew rapidly and in 1967 became autonomous from SEATO. Six years later it moved to its present rural setting in a 400 acre campus donated by the Thai government.

Educationally the main emphasis is on a five term master's degree in engineering or science, but PhD's are also an important part of the range of qualifications AIT can offer. At present the institute has 500 students and 62 full-time teaching and research staff. In both cases the range of nationalities is huge but most students are from Thailand itself or neighbouring countries such as Malaysia, the Philippines, Pakistan, India and Bangladesh. 60% of students who receive an AIT qualification return to their own countries to work in industry, government or universities. The institute clearly supplies a 'local' need.

Recently AIT has been gradually shifting from a strict commitment to infrastructural engineering to new fields which directly contibute to the porcess of national development in a Third World context. Areas such as food production, resources management, rural, industrial and urban development have become major concerns. The Divisions of Human Settlements is particularly active in this area and I spoke to Paul Vorratnchaiphan, a research associate, about one of the division's main interests, the Building Together Project.

The continual migration of rural folk to urban areas creates many problems for developing countries. To house people properly will require considerable changes in current low-income housing policy and, consequently, research into developing appropriate low-cost housing technology is needed. AIT is currently supervising a slum resettlement programme involving the rehousing of 180 families at Lad Phrao on the outskirts of Bangkok. The initial land purchase, development and coordination was financed by 'Brot für die Welt', a German organisation, and SELAVIP, a 'popular' housing service based in Chile. By mortgaging the land and completed houses to the Thai National Housing Bank this initial 'seed' money should be sufficient to finance in serial fashion many similar projects.

The main structure of the houses is formed by special interlocking lightweight construction blocks designed by AIT and made from a rice husk aggregate in a cement and sand mix produced on site. The prospective owners, working in groups on clusters of 16-20 houses, manufacturing all the component parts themselves. Each group is broken down into teams making joists, doors, piles or casements, *etc.* Seventeen weeks of part-time work are required from start to finish and by this time many of the previously unskilled owners have learnt their first trade. It is hoped that this will provide some of them with a new source of income and give a better sense of purpose and cohesion to the community.

Other AIT projects include the development of a system using composted human and animal waste as a nutrient source for freshwater algae and, ultimately, fish such as *Tilapia.*

I also spoke to Robert Exell, Associate Director of the Division of Energy Technology. Renewable energy resources are another important focus for AIT's research activities. Drying of crops, fish and meat are all long known applications of solar energy and one recent development is a solar powered rice dryer. This simple device draws air heated by the sun through a closed drying compartment containing the grains. These are protected from the rain by plastic sheeting on a bamboo framework. Half a ton of paddy rice can be dried in one to three days.

The same group has also developed a solar powered refrigerator for storing labile medical supplies in remote areas. AIT, however, hopes that the unit can also be made to provide a source of income for these communities. For example, it could be used to preserve commercially grown flowers during the critical period between cutting and refrigerated export to Hong Kong. AIT is currently collaborating with a local university project aimed at introducing this refrigerator and flower culture into the economies of primitive hill tribes in the north.

I spent a day on the AIT campus and only managed to visit two research wings. In fact the institute has nine teaching and research divisions, these being agricultural and food engineering, computer applications, environmental engineering, geotechnical and transport engineering, human settlements development, industrial engineering and construction, water resources engineering and, the most recent addition, energy technology. Also, the institute is the home of the South East Asian regional computer centre, one of the best engineering libraries in South East Asia, a language and media centre (the language used by the institute is English) and the Regional Documentation Centre.

David Bousfield



AIT's low-cost, appropriate housing for slum resettlement

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. . . or does the West have too much control?

THE Asian Institute of Technology (AIT) advertises itself as a centre of excellence which "provides opportunities for men and women of diverse Asian nationalities to study and work together to seek, define, and test technological solutions to the common problems affecting the quality of life in Asia". This claim is indeed partly justified; but the institute's impact on Asia, as a whole, has been negligible.

AIT's approach to the problems of Asia is based on the belief that academic excellence can be integrated with teaching and research for appropiate solutions to Asian problems. This approach has largely paid off and AIT can justly claim that its graduates are tailor-made for Asia's needs and that as a post-graduate technological institute it has produced over the past two decades, a small cadre of technocrats that now occupies key positions in Asia's private sector.

On the research front too, most AIT projects are based on problems of practical importance relevant to Asia's development. In their own way, some of these projects, such as integrated farm study, a small scale fishery project, slum settlements and flood forecasting studies, have made significant contributions at local level.

However, from a general regional perspective, AIT's contribution to Asia seems almost insignificant. In 1977, for example, AIT graduates included one graduate from Iran, 7 from Malaysia, 12 from Pakistan, 20 from Sri Lanka and 49 from Thailand. This year, AIT's graduate output included one student from Iran, 9 from Malaysia, 11 from Indonesia, 14 from Pakistan and 16 from Sri Lanka. On this basis of graduate output almost every technological university in the UK can claim to contribute much more to the educational development of Asia than AIT. With the exception of human settlement development, research at AIT too has failed to have a regional impact.

Indeed, both from the point of view of education and research, the chief beneficiary of AIT's endeavours is Thailand. Over one-third of AIT Alumni are Thais. Of the 15 reserch projects started in 1978, nine were related directly to the needs and requirements of Thailand.

AIT has considerable difficulty attracting students. Universities and colleges in many Asian countries do not know of its existence and consequently cannot recommend their graduates to apply for its post-graduate training. Even when a student applies and is accepted, political reasons may prevent him from attending. Most students who can go abroad to study would prefer to go to the West than to AIT in spite of its excellent reputation.

One reason why AIT has not made a significant impact on the region is that, even after twenty years, it is still regarded as an alien body. Even in Thailand, it is looked upon as having been transplanted from the West. In other Asian countries too AIT is thought of as non-Asian. In Pakistan, Iran and Turkey it is hard to find science policy makers at the ministerial level who do not have some reservations about it.

Part of the reason why AIT is labelled as an alien institution is that it is administered and funded largely by the West. Less than half of its 39-member Board of Trustees are Asian, and most of these are Thias with a token Malaysian, Indian, Indonesian and Sri Lankan. Amongst AIT's major backers are IBM, Shell, Esso and Bes Engineering. Other major contributors include Canada, Japan, US, Thailand and the Ford, Rockefeller, Chase Manhattan, Readers' Digest and Starr Foundations.

On the whole, the donors keep a good eye on the institute. Visits by their representatives are frequent. Indeed, as Dr Robert Excell, Associate Director of Energy Technology Division, who has been at AIT almost a decade says, some donors tend to dictate and influence AIT's policy. This trend seems to be increasing and, according to Excell, is a major area of concern for AIT's future. "We must try and remain independent of donor influence in the future", he says.

Donor influence apart, a major future area of AIT's contribution to the region is specialised science information. AIT has an excellent Regional Documentation Centre which was set up in 1977 with the objective of improving access to information in the region and of initiating and participating in information projects on a regional basis. There are also four specialised information centres that have already proved their worth: the Asian Information Centre on Geotechnical Engineering, the International Ferrocement Information Centre, the Renewable Energy Resources Information Centre and the Environmental Santation Information Centre. AIT's Regional Information Centre, together with the four special information centres, could act as a regional focal point for the global information system proposed by UNCSTD (*Nature* 6 September page 4), if AIT can seek out users and meet their needs.

Ziauddin Sardar

US strengthens scientific links with Latin America

Closer scientific links between the US and various Latin American countries are expected to follow a visit made last month to four countries by a top level team of science policy makers, headed by the President's science adviser, Dr Frank Press.

The delegation stressed that the purpose of the visits was directly in line with the agreements reached at the United Nations Conference on Science and Technology for Development in Vienna in August. "I was very pleased with the trip — the response was very warm and positive", Dr Press told *Nature*.

The visit also helped to prepare the ground for US strategy at the annual meeting of the Organisation of American States, which opened in Bolivia recently, at which the US is keen to promote technological and economic cooperation between Latin American countries as a way of promoting political stability on the entire American continent.

During the visits to Peru and Barbados, for example, the US delegation met with representatives of the two regional organisations, the Andean Pact, and the Economic Pact between Venezuela, Ecuador, Peru, Colombia and Bolivia — and the Caribbean Community Secretariat (CARICOM).

During the visit to Peru, the delegation met officials of the Junta del Acuerdo de Cartagena, the secretariat for the Andean Pact, to emphasize its willingness to support research programmes initiated by the Junta aimed at increasing technical collaboration between the five countries (see *Nature*, 12 October 1978).

The delegation's visit to Venezuela was aimed primarily at strengthening scientific links which already exist with the US. Particular attention was paid to proposals from the Venezuelan side on developing cooperation in basic and applied scientific research, particularly in the fields of marine biology, neurobiology, electronics and hydrology.

In Brazil, it was more a question of re-building links that have been strained in recent years. Here the specific topics under discussion included coal liquefaction technology, long-distance energy transmission by superconductors, and establishment of a diagnostic reference centre, as well as industrial technology and inter-university science and technology cooperation.

During the visit to Barbados, the delegation met with representatives of other regional development and research organisations. A communiqué issued after the meeting said that "the two sides expressed satisfaction with the progress made in the limited time available towards establishing a sound basis for science and technology cooperation between the United States and the Carribean". □

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correspondence

Human ingenuity is wasted on nuclear power

SIR,—I rather hoped that "nuclear nonsense" might draw a response from UKAEA. It is not nonsense that fast reactors produce 60 times more energy than thermal reactors. I said in my original letter (13 September, page 98) that it might be true in 200 years' time. Cutts and Smith (18 October, page 522) admit that it could not come about until after all thermal reactors had been replaced by fast reactors, and that on the best figures UKAEA could produce the ratio would only be 1.7 by 2020; so as they do not give a figure of their own, I take it that 200 years is not unreasonable.

What is nonsense is the use of this hypothetical long term ratio of 60 as an argument for developing fast breeder reactors now. It is all the more nonsensical because Granger and Merrick showed that even a delay in starting a fast breeder programme of as much as 30 years would be rapidly made up, because the main limiting factor is the production of plutonium by thermal reactors.

This is implicitly admitted by Cutts and Smith, because having predictably taken my "nuclear nonsense" to refer to the 60 times ratio, rather than Smith's use of it to support the development of fast breeder reactors, they then drop this argument and concentrate on lauding the possibility of eventually reducing uranium imports to zero. On this basis coal would seem to be a clear winner for the medium term, and the long term superiority of conservation plus solar energy over both hardly needs demonstrating.

It is an amazing paradox that nuclear scientists and technologists — even Alvin Weinberg (4 October, page 335) — can claim that human ingenuity is so great that it can deal successfully with all the problems which are associated with Three Mile Island and the 'China Syndrome', in a world dotted with nuclear reactors and repositories of highly radio-active waste requiring protection for thousands of years, a world in which the use of nuclear energy will inevitably be accompanied by the widest proliferation of nuclear weapons. Yet when faced with the job of utilising just one hundredth of one per cent of the energy showered on us by the only safe nuclear power plant — the Sun — human ingenuity flies out of the window, and only the difficulties can be seen. Yet this latter use of human ingenuity is the only one which offers even a glimmer of hope for the future of mankind.

Yours faithfully,

J. W. JEFFERY

Birkbeck College, University of London, UK.

Aflatoxin B_1 in barley in the UK

SIR,—In the United Kingdom in the early 1960s a link between the death of 100,000 turkey poults and the fungus *Aspergillus flavus* was recorded. Further studies identified the toxic entity of the fungus to be aflatoxin B_1, produced in imported groundnut. Further research led to the detection of aflatoxin in many animal and human feeds imported into the United Kingdom, e.g. walnut, pistachio, maize, cotton seed, etc.

Though *A. flavus* has been isolated in the UK, it has been thought either that strains are not capable of producing aflatoxin B_1, or that the substrate and conditions are not conducive to aflatoxin B_1 production. In June of this year, however, a farmer requested advice from Microbiology Department of the Ministry of Agriculture, Wales on the health risk to himself or his stock from mouldy barley. Enquiries found the barley had been treated with commercial propionic acid at an inadequate application level. Further laboratory examinations showed the mould to be *Aspergillus flacus* (5×10^7 prepagules/g barley), while mycotoxin examination gave a level of 125 μg aflatoxin B_1/kg barley.

The detection in a home grown cereal of aflatoxin must be regarded with some concern, because of its known carcinogenic and toxic properties. Also, questions must be asked as to the significance of a chemical additive. Additional studies are to be carried out to place this finding in perspective. We intend to study *A. flavus* isolates from soil and grain in Wales, for their ability to grow in varying concentration of propionic acid, and also, to assess such isolates for aflatoxin B_1 production. The results should help to clarify whether this incident is an isolated case or part of a greater problem.

Yours faithfully,

A. HACKING
N. R. BIGGS

Welsh Office, Aberystwyth, UK.

Cooperation with the USSR

SIR,—I have read the letter from Lee Lorch (13 September, page 98) with great concern. He does not seem to realize that cooperation must be two-sided; to be accepted as an equal partner, the Soviet Union must treat all its nationals on an equal footing.

Admittedly, the West may lose as much as the USSR by reduction of cooperation. But what other way is there of protesting against the way that the USSR is treating its Jewish scientists?

Yours faithfully,

H. LIPSON

University of Manchester Institute of Science and Technology, UK.

Wartime research on nitrogen mustard

SIR,—Thirty seven years ago the late Major J. M. Moore of the USA and I carried out classified experiments on the influence of nitrogen mustard — then a secret compound — on the bone marrow stem cells. Dameshek[1], Gilman[2] and Harrison[3] have commented on work prior to 1945 as being classified but important in the evolution of the treatment of leukaemia and cancer. Colleagues in Melbourne have suggested that for historical reasons we should have our work carried out in 1942 de-classified and published.

During the first world war mustard gas exposure in man was found to be associated with leukopaenia. This was believed to be due to superadded infection; it was not recognised that it was due to a chemical effect on the bone marrow.

In 1942 we tested the effect of nitrogen mustard on the bone marrow. We selected guinea pigs and compared the cellular distribution in bone marrow in a series of control animals against that of a series of similar animals exposed to the vapour of nitrogen mustard. Red marrow from the upper end of the tibia was examined. We found both the cells of the myeloblast series and the lymphoblast series were highly significantly reduced in numbers. This information was supplied to both the Ministry of Supply, London and also to Washington at that time. It is interesting to note that at that time much doubt was expressed that lymphocytes actually developed in bone marrow. This work was done in my laboratory at the University of Melbourne.

The Australian Defence Research Laboratory recently told me that there was no difficulty in obtaining security clearance for this work which was now common knowledge — but they were not able to find the documents recording the research of the "Gorrill" chemical warfare research team.

Our experiments on chemical warfare agents were essential to the war effort both owing to the capability of the Japanese in chemical warfare and the world ignorance of the precise effect of these agents under tropical conditions as applied to New Guinea. Unfortunately some years after the war, a directive was made that all documents relating to this work by the "Gorrill team" — later under the direction of another commanding officer — were to be destroyed. The senior official who destroyed them told one of us he though he might have been destroying significant scientific material. Under security wartime arrangements neither of us had copies of this document.

Yours faithfully,

E. R. TRETHEWIE

Walter and Eliza Hall Institute of Medical Research, Victoria, Australia.

1. Dameshek *Blood*, **4**, 338, (1949).
2. Gilman *Science*, **103**, 140, (1946).
3. *Text Book of Medicine*, Harrison, p. 1787 (McGraw-Hill, New York, 1977).

Obstructionists in environment clothing

SIR,—After its sincere start, "The end of the age of Aquarius?" (20 September, page 168) has been brought about in part by the duplicity of some so-called "environmentalists", who are, in fact, merely obstructionists. The writer personally investigated an attempt by such a group to include in the Organic Act, governing the US Bureau of Land Management, the setting aside of several square miles of rich coal lands in southern Utah as a sanctuary for the side-blotched uta, *Uta stansburyensis*. This small, brown lizard, with its subspecies, is common in suitable habitat over most of the Desert Southwest, from western Colorado to eastern California and is by no means in danger of extinction. Only the alertness of a US Senator from Nevada thwarted the move.

Even the discovery of the snail darter as an endangered species appears to have been based on an effort to block the construction of the Tellico dam, and not *vice versa*. Thus do the over-zealous and ulterior motivated subvert worthy endeavour, with the result that the efforts of the reasonable and sincere are eventually largely nullified.

Yours faithfully,

S. RALPH AUSTIN

Grand Junction, Colorado

news and views

Mitochondria spring surprises

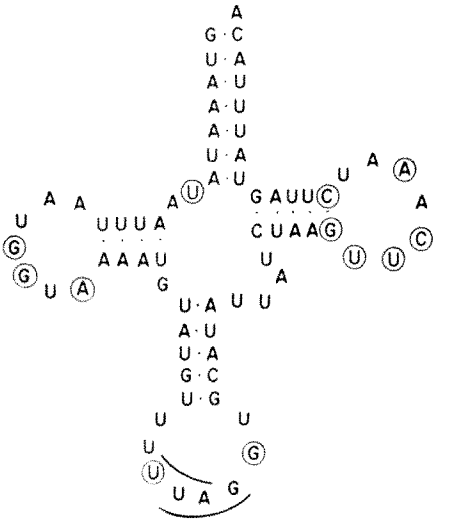
from B. D. Hall

THE concept of a totally universal genetic code was based intitially on studies of *E. coli*, tobacco mosaic virus, and human haemoglobin. The extrapolation of this conclusion from three genetic systems to all organisms was justified by the theoretical premise that any mutation which changed the ancestral code would cause so many different deleterious effects that it would have been selected against. Impressive though this argument may be, eukaryotic mitochondria have not heeded it. DNA-protein sequence comparisons for mitochondrial genes have revealed three variant codon assignments, at least one of which (UGA coding for tryptophan rather than acting as a termination codon) is used both in human and in yeast mitochondria.

The mitochondrial genome codes for a small number of proteins of the respiratory chain (eight in yeast) and contains genes for the tRNA and rRNA molecules of the mitochondrial translation system (Borst & Grivell *Cell* **15**, 705; 1978). Thus, as regards specification of the set of codons and anti-codons used, mitochondrial decoding of genetic information is autonomous and independent from that performed by the cytoplasmic system.

An extraordinary skewing of the mitochondrial codon usage pattern in favour of codons ending in A and U and away from those ending in G and C was evident from the first complete mitochondrial gene sequence determined, that for yeast ATPase subunit 9 (Hensgens *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 1663; 1979). For other yeast mitochondrial genes this same pattern holds, while for the single mammalian mitochondrial gene sequenced, only triplets ending in G are particularly infrequent.

The most surprising conclusion derived from the yeast subunit 9 gene sequence stems from an apparent conflict with the protein sequence (Sebald & Wachter, *29th Mosbacher Colloquium on Energy Conservation in Biological Membranes*, page 228; 1978). At position 46 the protein contains threonine, whereas the DNA sequence indicates a CUA codon (Hensgens *et al. op. cit;* Macino & Tzagoloff *Proc. natn. Acad. Sci. U.S.A.* **76**, 131; 1979), which normally codes for leucine. Follow-up sequencing studies established that the apparent discrepancy was due neither to a sequencing error nor to a genetic polymorphism. A structural basis for assignment of the CUA codon to threonine can be deduced from the sequence of an unusual yeast mitochondrial tRNA gene. Li and Tzagoloff have found (*Cell* **18**, 47; 1979) that a yeast mitochondrial DNA fragment which hybridises to $tRNA^{Thr}_{mt}$ contains a single tRNA gene sequence, that shown below



(From Li & Tzagoloff *Cell* **18**, 57; 1979)

This sequence differs from all previously-analysed tRNA sequences by having eight rather than seven nucleotides in the anticodon loop. Within this loop, the two nucleotide triplets nearest the centre are complementary to the nonsense codon UAA (overlined sequenced) and to CUA (underlined sequence). The mitochondrial tRNA transcribed from this gene may therefore be capable of accepting threonine and inserting it opposite the CUA codon triplet in ATPase subunit 9 mRNA and perhaps also at other CUA codons in mitochondrial mRNAs.

The second exceptional feature of the mitochondrial genetic code was discovered by similar protein — DNA sequence comparisons for cytochrome oxidase subunit II (COII), one of the three subunits of this enzyme which are made in mitochondria. The amino acid sequence of the COII protein has been determined for beef heart cytochrome oxidase (Steffens & Buse *Hoppe-Seyler's Z. Physiol. Chem.* **360**, 613; 1979), whereas the DNA sequences coding for COII have been identified in human and in yeast mitochondrial DNA. Barrell, Bankier and Drouin (this issue of *Nature*, page 189) have identified a sequence in human mitochondrial DNA which is colinear, identical in length and more than 70% homologous to the predicted COII coding sequence. Within this sequence there are three UGA codons in the translated reading frame. If we assume that the gene which has been sequenced does indeed code for human COII, it follows that there must be efficient read-through of UGA codons by the mitochondrial translation apparatus. This read-through is likely to be mediated by $tRNA^{Trp}_{mt}$ because each TGA in the human COII gene corresponds to tryptophan in bovine COII.

These strange goings-on in human cytochrome oxidase are not unique to our species. T. Fox of Basel University (*Proc. natn. Acad. Sci. U.S.A.*, in the press) has obtained very similar DNA sequence results for the yeast *oxi*1 locus, a mitochondrial gene which codes for yeast cytochrome oxidase subunit 2. The amino acid sequence of yeast COII decoded from the *oxi*1 DNA sequence, is 38% homologous to that of the bovine COII protein. This yeast gene, like its human counterpart, can only be translated to make COII protein if UGA codons are read as sense. Out of five TGA triplets in *oxi*1, four occur at positions where the bovine protein contains tryptophan. Similar conclusions were reached in an earlier publication which reported a partial DNA sequence for *oxi*1 (Macino *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3784; 1979). These authors raised the possibility that UGA also is read as a sense codon in two other yeast mitochondrial genes, *oli*2 and *box*4.

As a third possible variation, Barrell *et al.* (*op. cit.*) note that in mitochondria AUA may code for methionine, rather

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than isoleucine. From an alignment of the bovine COII amino acid sequence with the sequence of codons in the human COII gene, they observed that methionines occur opposite ATG at two and opposite ATA at five positions. Provided there was no large scale evolutionary interconversion of isoleucine and methionine codons it would appear that AUA codes for methionine in human mitochondria. There is not yet enough protein sequence data to determine whether AUA also codes for methionine in yeast mitochondria.

The structural grounds for the AUA to Met and UGA to Trp codon assignments are presumed by Barrell *et al.* to be nucleotide bases present at the wobble positions in the anticodons in $tRNA^{Met}_{mt}$ and $tRNA^{Trp}_{mt}$. The presence of U or modified U at the 5′ end of the anticodon in each case might allow translation of AUA and UGA by A-U base pairing and of AUG and UGG by wobble base pairing (Crick, *J. molec. Biol.* **19**, 546; 1966). Recent sequencing work by Sanger and Coulson (cited by Barrell *et al.*) has established that the anticodon of human $tRNA^{Trp}_{mt}$ has a sequence consistent with these postulates.

These variations of the mitochondrial genetic code from that which directs translation in *E. coli* illustrate that, once a genetic code came into existence, it was not immutably fixed in all its details. Will still more exotic variations in the code be found in other organisms? For the answer, consult the dictionary in your favourite dinoflagellate, protozoan or archaebacterium. □

Antimatter from space

from P.C.W. Davies

THE origin of matter is one of the great unsolved problems of science: the visible Universe contains about 10^{50} tons of material. Where did it come from? At one time it was supposed that all this cosmic stuff — from which the galaxies, the stars and our own bodies are made — always existed. Modern cosmologists, however, believe that the Universe is not of unlimited age, but came into existence about 15 billion years ago in the hot, dense, explosive eruption, popularly called the big bang.

If this primaeval explosion marked the creation of all the cosmic matter, physicists would like to understand the details: what precisely happened in the first microsecond of existence? Since the early 1930s it has been known that matter can be created from energy in the form of subatomic particles, so modern cosmology provides a natural mechanism for the origin of matter: it was created from the explosive energy associated with the primaeval violence. Paul Dirac gave a very heuristic explanation of how this happens. The equation that describes a particle such as an electron possesses two sets of solutions: one describing 'ordinary' electrons and the other mysterious states in which electrons have negative mass-energy. As all systems tend naturally to seek out their lowest energy, nothing apparently prevents ordinary electrons from descending into these enigmatic negative energy states. As the energies can be limitlessly negative, there is a sort of bottomless pit into which all matter can disappear amid a shower of energy.

To preclude this apocalypse, Dirac proposed that the pit is blocked with an infinity of invisible electrons. If one of these invisible negative energy electrons is given enough energy it will be promoted to a real, observable, positive energy electron. Thus, a new electron will suddenly materialise in space. Furthermore, the 'hole' left behind represents the absence of an invisible negative energy electron, which Dirac reasoned is equivalent to the presence of a visible positive energy anti-electron, or positron as it is now called. The positron is a sort of mirror image to the electron; like the latter, but with all its physical attributes (for example, charge, spin, magnetic field) reversed.

Positrons were discovered among cosmic ray debris in 1932, and since then, antiprotons, antineutrons and a host of other antiparticles have been identified. Until recently it was supposed that the creation of matter must always be accompanied by an equal quantity of antimatter of the corresponding type. This inevitably implies that for every atom of ordinary matter that we see, somewhere in the Universe lurks its opposite number. Because any contact between matter and antimatter results in the matter falling back into the 'holes' and disappearing from the Universe, it must be supposed that somehow the matter and antimatter have got themselves well separated. It is fashionable to suggest that there are whole galaxies made of antistars and antiplanets and, one may conjecture, antipeople. An antigalaxy would look, on the face of it, indistinguishable from an ordinary galaxy.

When matter and antimatter annihilate each other their mass gets converted back into energy in the form of gamma rays, so by measuring the flux of gamma rays from space, limits may be placed on the amount of mixing that occurs between the two species of material. This seems to imply that almost complete separation has occurred, even on the scale of galactic clusters. Presumably they were put asunder in the earliest epochs of the big bang but just how this could have happened is still not completely clear.

In the past few years an exciting alternative picture has emerged. Ambitious new theories that seek to unify three of nature's four forces into a single mathematical description have predicted that perhaps matter can be created without an exactly compensatory quantity of antimatter. If this is so, it opens up the possibility of a lopsided Universe in which most of the antimatter has been annihilated, leaving a small residue of unbalanced matter that constitutes all the observed Universe.

To test which of these theories is closest to the truth, physicists can search for antimatter in space. Although the nearest antigalaxy may be millions of light years away, perhaps high energy subatomic antiparticles can stream across the gap and pepper the Earth. Cosmic ray showers have long been known to contain some antimatter (that is how the positron and antiproton were first discovered), but it has not hitherto been known whether these are merely by-products of high energy collisions between ordinary particles among the cosmic rays and the Earth's upper atmosphere. Now an experiment has been performed by a group of physicists from Texas and New Mexico to search for antiprotons among the primary rays (*Phys. Rev. Lett.* **43**, 1199; 1979). From a high altitude balloon flight last 21 June, the group detected 46 such particles, of which only about 18 are attributable to reactions with the atmosphere.

The ratio of antiprotons to protons coming from space works out at 5×10^{-4} in the energy range explored, which accords well with theoretical estimates based on the assumption that no external flux of antiprotons is bathing the Galaxy. They can all be accounted for as by-products of collisions between ordinary cosmic rays and the tenuous interstellar medium in deep space.

This experiment not only provides important information for understanding the origin and behaviour of cosmic rays in the Galaxy, but it tightens up further the evidence against a matter-antimatter symmetrical Universe. If matter really can be created and annihilated alone, it will open up whole new chapters in both cosmology and subatomic physics. □

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100 years ago

In his last report from Saigon, Mr. Consul Tremlett alludes to his having been ordered by the Foreign Office to procure and send home a quantity of the bark known as *hwang-nao*, which during the past four or five years has been exported from Tongking to Trinidad, and there seems to have proved efficacious in cases of leprosy. The tree from which it is obtained is hardly known except to the missionaries, and is only found in the mountain forests of the north of Annam.

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The road to unification

Sheldon Glashow, Abdus Salam and Steven Weinberg have won the 1979 Nobel Prize for physics for their great contributions to a unified theory of two of the fundamental forces of nature — the weak and electromagnetic interactions. But the theory took a long time to develop, and in that time many physicists other than those who were awarded the final accolade also made important contributions to it. On the next page Robert Walgate outlines the background of the theory and Norman Dombey traces here, for the initiated, the history of the Glashow-Salam-Weinberg model.

THE award of the Nobel prize in physics last week to Gashow, Salam and Weinberg can be seen as marking the end of the second phase of post-war theoretical particle physics. The first phase, which lasted until the mid-1950s was concerned with the development of quantum electrodynamics (QED) and is associated with the names of Tomonaga, Schwinger, Feynman and Dyson. The second phase begins with the discovery of 1957 of the non-conservation of parity in weak interactions — the interaction responsible for the neutron beta decay $n \rightarrow pe^-\bar{\nu}$ and muon decay $\mu^- \rightarrow \nu e^-\bar{\nu}$ — following its prediction by Lee and Yang[1].

After this, there was rapid progress in the understanding of weak interactions. Fermi[2] had written down a contact coupling of the four fermions involved in beta-decay in 1933 and for over 20 years it was the task of experimental nuclear physicists to work out the details of that coupling. The parity experiments then fixed the Fermi coupling as a current-current coupling of the neutron-proton or muon-neutrino current to the electron-neutrino current. (Current here has the technical meaning of behaving like a vector plus axial vector under Lorentz transformations.) Furthermore, the experiments showed that the strength of the vector part of the Fermi coupling G_v for beta-decay was almost precisely the same as G_μ for muon-decay.

A possible explanation for this was given independently by Gershtein and Zeldovich[4] and Feynman and Gell-Mann[3]. If the vector current of weak interactions were conserved, irrespective of whether it involves the strongly-interacting hadrons (nucleons, pions and so on) or only involves the leptons $\mu^\pm$, $e^\pm$, ν which have no strong interactions, then as in electrodynamics there is an associated conservation law. So in place of charge conservation, which leads to the equality of the electric charge of the proton and e^+, there is the weak 'charge' equality $G_\mu = G_v$.

Once this was accepted as a serious possibility, there was a clear analogy between the electromagnetic and weak interactions as both involved a conserved current and both were universal in that they involved leptons and hadrons with equal strength. There are of course many important differences: the characteristic strength of weak interactions is given by G_V where $G_V m_p^2 \approx 10^{-5}$ (m_p is the proton mass) whereas the equivalent strength of electromagnetic interactions is given by the fine structure constant $\alpha \approx 10^{-2}$; electromagnetic interactions conserve parity whereas weak interactions violate it maximally; electromagnetic interactions are long range whereas weak interactions act at a point; and so on. Using the analogy, Schwinger[5] in 1957 wrote down a unified weak-electromagnetic interaction based on the Yang-Mills[6] generalisation of QED. This involved a triplet (γ^+, γ°, γ^-) of charged photons: the theory conserved both charge and isotopic spin and thus provided a dynamical basis both for isotopic spin conservation and for the connection between charge and isotopic spin. Unfortunately, no zero mass charged photons have ever been seen.

Nevertheless, Yang-Mills theory does lead to a conserved vector current with charged components and that is precisely what is required for weak interactions. Schwinger had to assume that there was some mechanism by which the charged photons $\gamma\pm$ acquired a very heavy mass so as to simulate the pointlike character of the weak interaction: these were to become known as the intermediate vector bosons $W^\pm$ of the weak interaction. If this mass M_W satisfied $G_V M_W^2 \sim \alpha$, that is $M_W \sim 30$ GeV, then the resultant weak-electromagnetic interaction was unified in the sense that now α represented the coupling strength of both interactions. From this viewpoint, the reason that weak interactions were so weak was simply because the $W^\pm$ were so heavy that they were unlikely to be exchanged at the energies characteristic of nuclear physics. The properties of this theory were further developed by Schwinger's student Glashow[7] and Salam and Ward[8] during 1958.

Not many physicists took these early papers seriously as the parity-violating aspects of the charged weak current were completely unexplained and difficult to reconcile with the parity-conserving electromagnetic current. The first paper which made real progress was published by Glashow[9] in 1961 and provides the foundation of the present theory. Glashow showed that the parity properties of the electromagnetic and weak currents were possible to reconcile in terms of the known leptons provided that a triplet ($W^+W^\circ W^-$) of vector bosons coupled to the left-handed leptons while a fourth vector boson B° coupled to both left and right-handed leptons. Then quantum mixing between B° and W° gave rise to the physical states — the photon γ and a predicted neutral vector boson Z°. In this way it was possible to arrange that the photon coupled equally to left and right handed leptons thus conserving parity whereas Z° coupled unequally. So in this SU(2) × U(1) theory (the technical name of the underlying group), there was a new neutral weak current coupling to Z° which violated parity in a predictable non-maximal way. Glashow's work was followed in 1964 by an independent derivation of essentially the same result by Salam and Ward[10], who surprisingly were unaware of Glashow's work. The main difference between the two approaches is that Glashow has two left-handed doublets (ν_e, e^-), ($\nu_\mu\mu^-$) to describe the leptons whereas Salam and Ward preferred the group SU(3) and took the basic lepton triplet (μ^+, ν, e^-) with a 4-component neutrino instead of two 2-component neutrinos ν_e, ν_μ. Salam and Ward's choice allows certain possibilities absent for Glashow such as a neutrino magnetic moment and *vice versa*, but there is no necessary difference between the theories.

These early papers give most of the results of the present theory when applied to leptons. Nevertheless, the theories were not renormalisable: unlike in QED it was not possible to calculate higher order effects in the coupling constant α. The problem of calculating with massive vector bosons (that is spin -1 particles) had been known for many years. Such particles can have their polarisation vector either perpendicular (transverse) or parallel (longitudinal) to their direction of motion. A zero mass, spin -1 particle can by Lorentz invariance only have transverse modes but a massive vector boson must have the longitudinal mode too. The problem arises because the longitudinal polarisation vector has a component proportional to the momentum vector k_λ; hence the probability of emitting (or absorbing) the particles in their longitudinal mode will increase both with particle momentum/energy and with

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the number of particles emitted. This is not possible in a sensible renormalisable theory which should give finite results at all energies and for any number of particles. In renormalisable theories all processes to any order in the coupling constant are calculable in principle.

Salam [11] in particular emphasised the importance of the renormalisability criterion and thought that it should follow as in QED from a principle of gauge invariance. In QED this is the statement that the components of the electromagnetic field vector A_λ in the direction of the photon momentum k_λ are redundant. It follows just as in classical electrodynamics that the photon (or electromagnetic wave) can only possess transverse modes, thereby removing the difficulty inherent in longitudinal modes. However, for QED to be gauge invariant the photon must have zero mass. Yang-Mills theory involves a more complicated gauge invariance where the 'photons' must also have zero mass.

The problem of inducing a vector boson mass without destroying the gauge invariance of the theory was actually solved in 1964 in some seemingly obscure work based on the theory of superconductivity. In superconductivity the ground state of the system is not invariant under the full symmetry of the interaction describing the system (a simpler example is ferromagnetism where the direction of the ferromagnet in its ground state clearly breaks the rotational symmetry of the underlying spin-spin interaction); an energy gap thus appears between the ground state and the lowest symmetric state. Nambu and Jona-Lasinio[12] showed that this process of spontaneous symmetry breaking (SSB) can be considered as the generation of mass for a fermion initially with zero mass but with a pairing interaction as in superconductivity. The energy gap behaves like a mass.

Brout and Englert[13] and Higgs[14] then showed that if one starts with a gauge theory involving a zero mass vector boson interacting with scalar fields, then SSB will generate a mass for the vector boson without destroying the gauge invariance of the underlying interaction. So it is possible that the good (that is, renormalisable) properties of the underlying theory are retained. In this case at least one scalar particle must also exist as a result of SSB.

It is surprising in retrospect that it took physicists three years to incorporate the ideas of SSB into unified weak-electromagnetic theory. In 1967 Weinberg[15] applied these ideas to Glashow's $SU(2) \times U(1)$ theory thereby predicting the mass relation $M_W = M_Z \cos\theta$ where θ is Glashow's mixing angle so giving the strength G' of leptonic neutral currents as $G' = \frac{1}{2}G_\mu$. Furthermore, the mass M_W is $37/(\sin\theta)$GeV or about 78 GeV with the present value for $\sin\theta$. Salam at about the same time was applying the ideas of SSB to his own and Ward's version of the theory. His results[16] are in agreement with those of Weinberg but his more general approach did not allow him to derive explicit mass relations for M_W and M_Z.

The rest is history. On the theoretical side, Glashow, Illiopoulos and Maiani[17] in 1970 introduced the concept of charm (*Nature* **262**, 537; 1976) thereby allowing the unified theory to be applied to hadrons and not only to leptons; the following year 't Hooft[18] showed in a *tour de force* that the Weinberg model was renormalisable. Experimentally, weak neutral currents were discovered in the remarkable experiment[20] at SLAC last year (*Nature* **274**, 11; 1978), when it was shown that left-handed electrons scatter off deuterons more than right-handed electrons.

In conclusion it is only fair to give credit to those whose work was inextricably linked with the development of the theory, which as is clear was very much a

What the theory is about

EINSTEIN spent the latter half of his life struggling to find a unified theory of the gravitational force and electromagnetism; he failed because he could not transfer the geometrical ideas of the general theory of relativity (his space-time theory — still the current theory — of gravitation) to the electromagnetic field. However he twisted and turned his electromagnetic 'vectors' and 'tensors' electromagnetism remained stubbornly non-geometrical: the electric and magnetic forces were something you simply had to add to space-time, like actors on a stage.

But now Glashow, Salam, and Weinberg, and the physicists who worked before and with them (see accompanying article) have turned the tables on Einstein by unifying electromagnetism with something else — the weak interaction, which if is responsible for the 'beta decay' of radioactive nuclei and for the transformation of protons into neutrons (and hence hydrogen into helium) that allows the Sun to burn. Both electromagnetism and the weak force are described in the theory as fields of force laid upon space-time; but they are now conceived as aspects of the same field of force, seen in different circumstances, just as magnetism and lightning are different aspects of the electromagnetic field.

Electricity and magnetism, incidentally, were unified by the Scot James Clerk Maxwell towards the end of the 19th century, in a 'field theory' which is still very much at the root of modern theories of fields of force. Maxwell showed that there must be propagating waves of electromagnetic field, and that they would travel at the speed of light; Hertz demonstrated their existence.

Light is an electromagnetic wave radiated by moving electric charges. It follows that if the weak force is to be combined in a unified theory with electromagnetism, there must be 'weak' charges and a corresponding radiation. In particle language (corresponding to the invocation of quantum mechanics, and the 'quantisation' of the field) there are photons (for light) and what came to be known as 'intermediate vector bosons' for the weak radiation. The difference is that photons are massless, and can cause interactions at infinitely long range, whereas the IVBs are massive and cause short-range forces. Thus the weak field of protons and neutrons cannot be felt outside the atomic nucleus whereas their electric field extends outwards and captures electrons, thus creating atoms. Inside the nucleus, the weak field causes beta dacay.

So far so good, but we still only have parallel theories, not unified ones. The next step was highly technical, and took a long time to emerge, but has very interesting consequences. It proved that the weak and electromagnetic fields could be understood as aspects of the same field only if the 'uni-field' was being propagated though a physical medium, the Higgs medium spreading throughout space-time. The medium was rather like calcite, which splits light into two beams according to its polarisation; the Higgs medium splits the uni-field (which has massless, long-range particles) into a heavy part (the IVBs) and a massless remainder (light).

Back to the aether, do I hear? Not quite. The medium is supposed to be real and detectable, and it obeys all the constraints of special relativity. Since it is uniform throughout space-time it is not noticeable until it is altered locally in some way, which cannot be done except at very high particle energies; but at those energies it is possible to predict that 'Higgs particles' will be created, like splashes in the medium (they will correspond roughly to density waves like sound in air).

The theory also predicts the exact masses and charges of the IVBs (there will be three, positive, negative, and neutral); and although it has been well verified at low energies in experiments with electrons and neutrinos, the real test will come with the detection (or not!) of the IVBs and the Higgs particles. Meanwhile particle theorists are attempting to apply the techniques of Higgs media (or 'spontaneous symmetry breaking' as it is called) to a unification of the weak, electromagnetic, and strong forces — without, yet, it must be admitted, much success.

Once the experiments that test the Glashow-Salam-Weinberg model have been done we shall know whether we are on the right lines or whether we have to start along a completely new track.

Robert Walgate

collaborative effort, but whose names were not chosen at Stockholm: in particular Ward and 't Hooft. It is also necessary to add the note of caution that so far neither the vector bosons $W^{\pm}$ and Z^0 nor the scalar particle implied by SSB have been discovered (*Nature*, **279**, 675; 1979). If the mass of the Z^0 is indeed 90 GeV as predicted, then it will be discovered in the next two or three years at PETRA in an e^+e^- experiment and at CERN in a $\bar{p}p$ experiment. If not . . . □

Small-scale solar magnetic fields

from Lawrence E. Cram

OBSERVATIONAL studies of the solar atmosphere show that a large fraction of the magnetic flux penetrating the solar photosphere occurs in elements with field strengths in excess of 1 kG. The elements range in size from large sunspots with diameters exceeding 20 Mm, down to tiny flux tubes whose diameters are inferred to be smaller than 100 km. The occurrence of strong fields in discrete structures of this kind has important consequences for our ideas concerning the generation, evolution and decay of solar magnetic fields, the structuring of the solar photosphere, chromosphere and corona, and the origins of non-radiative heating and mass transfer in the solar atmosphere. The observed properties and theoretical consequences of highly structured, intense solar magnetic fields were discussed at a recent meeting held at Sacramento Peak Observatory (SPO).*

J. Harvey (Kitt Peak National Observatory), summarised the current observational picture of small scale magnetic fields. Magnetograph observations of the net flux in areas greater than about $(2 \text{ arc s})^2$ show that in the quiet Sun almost all flux is concentrated in 1-2 kG 'bundles,' which occupy only a small part of the observed area. The area occupied by the stong field increases with increasing height, as the field flares out. In the general neighbourhood of the area where the flux elements penetrate the photosphere, there is a perturbation in the photospheric brightness distribution (called filigree or facular elements) and in the absorption line spectrum (line gaps); however, there is not a one-to-one spatial correspondence between the magnetic and brightness structures. This implies that there is not a tight temporal correlation between the evolution of brightness structures, which change in a few minutes and magnetic structures, whose evolution has not yet been studied with adequate spatial resolution.

The correlation between magnetic and velocity fields in the flux bundles is a perplexing problem. Many observers have found that the material in the magnetic elements is flowing downwards with a velocity of the order of 1 km s^{-1}. In a careful study, Giovanelli and Slaughter (*Solar Phys.* **57**, 225; 1978) showed that there is a downdraft whose amplitude increases with depth, highlighting the following problem: if the conductivity of the solar atmosphere is as high as is universally assumed, the field lines should be frozen into the plasma. If this is the case, whence comes the large mass flux that is continually flowing down the magnetic elements? Avoiding the problem of the origin of the downdrafts, E. Parker (University of Chicago) showed how the magnetic elements could be cooled, and thereby maintained in concentrated form, by such flows.

During periods of good seeing, the extremely sensitive Kitt Peak magnetograph reveals small-scale bipolar structures almost everywhere on the solar surface. These are distinct from the small-scale intense flux tubes that occur predominantly in the photospheric network, and are called the inner network field (INF). The properties of the INF are not well known. As A. Title (Lockheed) reported, a magnetogram obtained at SPO with 0.5 arc s spatial resolution does not reveal an INF component with a rms field strength exceeding 50 G. The possible existence of a chaotic magnetic field with a strength of a few gauss, pervading the entire solar photosphere, has great theoretical implications, and the demanding observational problems of high sensitivity and superb spatial resolution will be attacked by observers in an effort to tie down the properties of the INF.

Parker stressed the vital importance of solar studies as the foundation for astrophysics. "If we don't understand the relatively well-observed MHD phenomenon of sunspots, how can we have confidence in speculations about MHD and plasma processes in such objects as quasars and galaxies?" he asked. Parker described the physical basis of several characteristic properties of small-scale intense magnetic flux tubes, and highlighted some of the basic theoretical problems posed by the existence of small-scale magnetic structures and sunspots. His recent sunspot model (*Astrophys. J.* **230**, 905; 1979) was discussed, but the general consensus among observers and theoreticians alike was that the formation, evolution and decay of sunspots are, in most respects, unsolved problems.

N. Weiss (University of Cambridge) discussed the connection between surface magnetic fields and the magneto-gas dynamics of the solar interior. He emphasised the importance of flux emergence in ephemeral active regions (EAR, closely related to X-ray bright points). When these are taken into account, the rate of flux emergence through the solar photosphere is essentially constant through the solar cycle (about 3×10^{22} Mx per day), and is sufficient to replace the surface flux every 10 days. This fact, together with the fundamental property of solar magnetic fields to appear always in the form of strong-field flux tubes, poses tremendous problems for the theory of the cyclic generation, transport and reversal of solar magnetic fields by dynamo action.

The most advanced existing dynamo models are based on mean-field electrodynamics, but the existence of intense, inhomogeneous solar surface fields strongly suggests that mean field theory is inappropriate. Weiss suggested that a more realistic picture of the solar interior would involve a constantly changing framework of magnetic structures with convective eddies lying between them. Any description of dynamo action in such an intermittent magneto-convective region would involve a 'flux-tube dynamo.' Weiss sketched a model of this kind, involving field generation in a zone of penetrative convection between the solar radiative core and convective envelope. Such a model has the advantage of providing a relatively ordered field by avoiding the inevitable 'tangling' by convection acting over a time scale of years, and it also removes one of the major problems of flux-rope theories, that magnetic buoyancy in the convection zone forces flux to the surface too rapidly.

It is interesting to note how the changing observational picture of solar magnetic fields has vindicated many of the objections to conventional theories made by J. Piddington (CSIRO: see *Astrophys. Space Sci.* **55**, 401; 1978). Piddington has been frequently criticised for not providing a 'rigorous mathematical treatment' to support his ideas, but as he pointed out, "no treatment is acceptable, let alone rigorous, unless it is based first on adequate observational data and second on sound physical interpretation." Probably our understanding of solar magnetic fields will improve with the melding of Piddington's picturesque models with the powerful mathematical methods being developed to study intermittent MHD turbulence.

In summarising a number of important observational problems that emerged from the meeting, C. Zwaan (Utrecht) emphasised the need to study in detail the physical processes occurring in single small flux tubes. Such studies require sensitive velocity and magnetic field measurements to be made over relatively long periods with excellent spatial resolution. The 1.25 m Shuttle Optical Telescope (SOT) designed by R. Dunn (SPO) will provide the technological leap required to answer problems concerning solar atmospheric structures on the 100 km scale. Other important problems may not need such new instrumentation, but rather the careful application of existing equipment. One fascinating question is "how does

*A summer symposium on Small-Scale Solar Magnetic Fields was held at Sacramento Peak Observatory on 16-21 July, 1979.

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magnetic flux disappear from the solar photosphere?''

There is a growing body of evidence pointing to the widespread existence of stellar chromospheres, coronas, and winds, all of which seem to be variable and many of which may be related to 'magneto-gas-dynamic processes' in stellar atmospheres. A sobering conclusion of the meeting was that we must do much more work to understand the intricacies of solar magnetic fields, and it will be a long time before a satisfactory account of stellar and cosmic electrodynamics is available.

Rapid screening of carcinogens — end of the beginning?

from Philip D. Lawley

THE problem of the aetiology of cancer is now of no mere academic interest, but of considerable socioeconomic importance, in the face of increasing public scepticism regarding effective prevention or treatment.

Recent recognition of the importance of rapid screening for potential carcinogens (almost synonymous with testing for potential mutagens according to methods devised by B.N. Ames) followed from the hope that elimination of these chemicals from the environment would lead to a significant reduction in cancer incidence. However, this optimism seems to be drifting away, as, for example, estimates of the occuational component of cancer incidence first increased, from their generally accepted lowish level (1-5%), to a forecast of 23-38%, followed by a reaction against the higher figures.

This sense of scepticism is not confined to epidemiologists and cancer aetiologists. If informed current opinion is represented by such a respected literary figure as Susan Sontag (in her recent book, 'Illness as Metaphor'), then both historically predominant themes in cancer aetiology, the 'environmental-mutagenic-chemical' and the 'hormonal-developmental-immunological', are dismissed, and *the* cause of cancer is maintained as an unsolved mystery. (These themes can be traced back, for the latter school of thought, to observations on proneness to breast cancer of 'melancholic' as opposed to 'sanguine' women by Galen (2nd century AD), or of nuns (Ramazzini, 1700); or, for the environmental school, to those on chimney-sweeps' cancer by Pott (1771)).

The more recent 'somatic mutation' theory originated a mere hundred years ago, and found biochemical support from observations that the alkylating agent, mustard gas, later established as a carcinogen in man, reacted with nucleic acids *in vivo*, a finding subsequently extended to virtually all classes of chemical carcinogen.

The invaluable 'Ames test' (in reality a mini-battery of tests using bacteria as the test organisms) permits rapid bioassay of chemicals, or their reactive *in vitro* metabolites, for mutagenic activity. However, to validate this test against many known carcinogens (established through conventional animal tests), it has been necessary to strip the bacteria of their DNA repair capability, and to sensitise them further by introduction of a mutator plasmid. The *in vitro* metabolic activation system (from liver of rats) rarely, if ever, mimics exactly the metabolism of carcinogens *in vivo*, even in rat liver, itself rarely the target tissue.

Furthermore, a fundamental difference exists between the DNA repair capability of bacteria and that of mammalian cells, which has been recognised from the earliest comparative work. Mammalian cells can reproduce aparently quite normally, as judged by survival of colony-forming ability and lack of induced mutation, despite incomplete removal of chemically (or radiation) induced DNA damage. Whether this indicates more effective repair (the remaining damaged DNA being redundant), or whether it is a source of further potential mutations, perhaps remains debatable. Nevertheless, mammalian cells, and in particular normal human fibroblasts, are remarkably resistant to low doses of mutagens or carcinogens, by these criteria.

The overall picture to the biochemist is, therefore, that any quantitative correlation between rapid screening data and the risk of human cancer from exposure to the test chemical is likely to be tenuous, *a priori*. Against this background, and with the now prodigious interest in, and expenditure on (at least in the US), this area of research, it was indeed appropriate that a conference* should be called to undertake a rigorous examination of this relationship. In addition, the associated fledgling topics of possible rapid screening methods for teratogens and nephro and neurotoxic chemicals were discussed, in the light of experience with the parent 'Ames tests'.

The topics covered in this blockbuster of a conference ranged from 'Molecular Mechanisms of Carcinogenesis' to 'Risk Assessment Approaches for Establishment of Criteria and Standards', culminating in a round-table discussion on 'Development of Environmental Policies', this last dominated by the eloquence of a named US Presidential candidate, B. Commoner (Washington University, St Louis).

On molecular mechanisms, the emphasis is shifting to recognition that while the intiation stage (somatic mutation) may be necessary for carcinogenesis, it is not of itself sufficient. Subsequent stages (denoted by some as 'promotion', somewhat to the dismay of P. Shubik (University of Heidelberg, an originator of the concept) may be at least as important, and there was much talk of 'synergism', 'cocarcinogenesis', and 'late stage carcinogens'. M. Radman (Université Libre de Bruxelles) thought that X-radiation had more of the characteristics of a promoter than of an initiator, and that promotion involved chromosomal rearrangement, but other speakers stressed the involvement of the interaction of promoters with cell membranes. Shubik questioned the tendency of some to attribute reversibility to the process of promotion.

With regard to the fundamental and over-ridingly important question of relevance of rapid-screening to the assessment of human cancer risk, I cannot do justice here to the thorough discussion of the topic that was given. As a UK taxpayer one remains uncertain whether shame at one's country's lack of expenditure on toxicology, or admiration of the US research effort in this area, should be the predominant emotion.

M. Waters (EPA, Research Triangle Park) showed convincing evidence from tests at various levels, and from epidemiological studies, particularly for a range of pesticides, that 'tier tests' were not an acceptable vehicle for assessment of cancer risk, and that a 'battery' of tests, including conventional animal life-time carcinogenesis studies, was notably less fallible. He held out some hope for a test based on transformation of human cells in culture, but the notorious difficulties already encountered seem to be in line with the known relative lack of susceptibility of normal human cells to carcinogens.

This indication also emerged from the thorough comparative assessments (by R. E. Albert (New York University) and others) of the results of quantitative animal carcinogenesis tests and human epidemiological studies, using the conservative assumption of linear dose-response at the low doses of environmental chemicals to which man is exposed. Generally man emerged as, at most, rather less susceptible to environmental carcinogens than the animals used in the conventional tests. From the reasonable estimates of the levels of environmental exposure, it seemed unlikely that these could account for a substantial proportion of the aetiology of human cancer. The current trend was expressed as 'downgrading of environmental causality'.

Another controversial issue concerned

*Nato Advanced Research Institute, Monaco, 22-28 September, 1979: *'In vitro Toxicity Testing of Environmental Agents: Current and Future Possibilities,* organised by A Kolber (Chairman) (Research Triangle Institute, North Carolina), L. Grant (US Environmental Protection Agency), S. Harry (US National Institute of Environmental Health Science) and J. McLachlan (USNIEHS) and T. Wong (Research Triangle Institute).

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the implications of results from rapid screening for regulatory policy. Previously (see *Nature* **278**, 388; 1979), President Carter's advisers at the US Office of Science and Technology expressed the view that "it is necessary to start by distinguishing scientific from regulatory decisions" — "scientific data collection and analysis" was one matter, but "cost benefit analysis" definitely another.

This view did not seem to be shared by B. Commoner, who began his presentation of results, from Ames tests of human body fluids and foodstuffs, by stating his belief that this type of experimental work could, in certain cases, supersede epidemiological studies, and did have direct implications for regulatory policy.

On the lighter side, he included an entertaining account of his work showing that red onions contain mutagenic flavonoids, leaving white onions, in the absence of any known specific nutritive value of their congeners, as winners in the risk-benefit analysis.

Some asperity developed between Commoner and R. Morgan (University of Toronto), who expressed the anguish of at least some of his fellow epidemiologists, that "the Ames test has led to more hypotheses than could possibly be tested", but, perhaps in some compensation, has been of distinct benefit to the legal profession. In an elegant presentation, Morgan showed that, in agreement with epidemiological experience, increasing the sensitivity of a screening test (certainly a marked feature of the history of Ames' tests) necessarily decreases its specificity and hence its predictive value.

The 'book of the meeting' will be required reading for many administrators (or indeed any of the public seeking a comprehensive view of this important area of research) as well as cancer aetiologists and toxicologists in general. □

The fascination of echinoderms

from Norman Millott

ECHINODERMS do things differently, which is one good reason why they have attracted increasing numbers of biologists with widely differing specialisations. This appeared to advantage in a recent colloquium*. Communications were so many and varied that only a few of the many excellent ones can be mentioned here.

Palaeontologists — seemingly tireless in their devotion to the group — were in part concerned with rare fragments of Jurassic echinoids from Western Australia indicating a wide and rapid spread of cassiduloids. Others challenged existing ideas concerning the primitiveness of the cidaroid lantern, or urged the need for a critical revision of classical changes in Adriatic echinoids to deepening of the sea, changes in climate and sediment and accumulation of calcium carbonate. D.G. Stephenson (Keele University) advanced yet one more idea concerning the seemingly eternal mystery of the five-rayed symmetry, showing that it could have conferred advantages in passive suspension feeding and could have been independently evolved in various groups of echinoderms.

Taxonomy, ever restless, was represented in the findings concerning holothurians of a 28-year survey of the Chinese coast, yielding incidentally, two new species and a proposed new genus. Other communications argued for regarding *Holothuria stellata* as a genuine species and for transferring the genus *Ophiocomina* to the Ophiacanthidae.

Ideas concerning the affinities and phylogeny of echinoderms invariably command attention. H. and G. Termier (Université P. et M. Curie, Paris) envisaged the evolution of coelomates as involving an early separation of echinoderms from lophophorates and chordates, with development of 'architectural' diversity and adaptive radiation in the Cambrian. R.P.S. Jefferies (British Museum (Natural History)) also painted a broad canvas, adding more detail to his picture of chordate evolution, deriving these animals from his palaeozoic Calcichordates, which show echinoderm affinities, yet possess what he interprets as tell-tale characters of chordates.

Echinoderms have nothing if not spectacular coelomic systems, achieving much by hydrostatic pressure, circulation of coelomic fluid and wandering cells. J. Binyon (Royal Holloway College, University of London) reported experiments determining the level to which fluid pressure could be raised in starfish before structural damage occurs, while C.W. Walker (University of New Hampshire) supported by direct observation, the importance of flagellated collar cells in producing coelomic circulation. Some functions of the ubiquitous wandering cells, including their bactericidal action, were elegantly demonstrated by L.I. Messer and A.C. Wardlaw (University of Glasgow) and a very different relationship with bacteria in *Leptosynapta* was reported by J-P. Feral (Museum National d'Histoire Naturelle, Paris) who described their constant association with cuticular structures. Others demonstrated the ultrastructure and symbiotic associations of amoebocytes, their relation to lesions in the body wall and their importance in transporting gut enzymes. The importance of the enigmatic 'haemal' system in moving assimilated substances, accumulating lipids and eliminating effete phagocytes was also indicated.

The extraordinary plasticity of peritoneal cells which Farmanfarmaian and I showed in 1967, received striking confirmation from A. Van Der Plas and M. Jangoux (Rijksuniversiteit, Utrecht & Université Libre de Bruxelles) who described the migration of these cells into the epithelial lining of the pyloric caeca of *Asterias* to form lipid stores showing changes related to the reproductive cycle.

Concerning the nervous system, there were reports of an unusual mode of dual innervation in echinoid podial muscles, of the importance of the radial nerve and its own sector of the nerve ring in the arm regeneration of *Asterina* and (for a change!) an analysis of associative learning in *Marthasterias*.

Physiological flexibility was the keynote of communications concerning rock pool species and the remarkable tolerance of sudden and extensive salinity changes of *Ophiura albida* in Loch Etive.

Much was said about nutrition, including the partition of energy between respiration, excretion and growth in plutei, and in starfish, changes in diet and population structure which occur with depth were reported, as well as the existence of a 'waiting stage' between larval and growing phases when food is scarce. Differing feeding processes in echinoids were correlated with differing fine structure of their radiole spines, and it was indicated that the shallow water holothurians of Enewetok Atoll do not occupy the same habitat unless they have different feeding habits.

Population dynamics with undertones of pollution and economics were represented in separate accounts of the effects of sewage sludge dumping near the mouth of the Elbe, where brittle stars were involved in the ensuing instability of the benthos, and of the possibility of introducing threatened edible species into suitable sites. Impressive densities of echinoderms off the west coast of Ireland and their dominant role in benthic economy were emphasised by a group of workers from University College, Galway. These were the contributions of professional scientists, but D. Nichols (University of Exeter) rightly paid tribute to the important contributions to his survey of threatened *Echinus*, made by amateur and professional divers.

As befits a modern programme, biochemistry and cytochemistry were represented by reports concerning the importance of steroids and the occurrence of gut enzymes in various species as well as the different aspects of the action of 1-methyladenine on starfish oocytes in which a calcium-binding factor resembling calmodulin was reported. □

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*A European Colloquium on Echinoderms, dedicated to L. Cuénot, was held at the Université Libre de Bruxelles on 3-8 September. It was organised by Dr. M. Jangoux, Laboratoire de Zoologie.

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AUTUMN BOOKS

Prometheus of the atomic age

Otto R. Frisch

Scientists in Power. By S.R. Weart. Pp.343. (Harvard University Press: Cambridge, Massachusetts, and London, 1979.) $17.50; £11.35.

IT is widely known that Enrico Fermi and his team started the first controlled nuclear chain reaction on December 2, 1942, in Chicago. This book tells us how a Frenchman, Frédéric Joliot, rather than Fermi, might have become the Prometheus of the atomic age, but for the German occupation of France. Madame Curie had picked the promising youngster for her assistant in 1925; she soon saw that he was a "skyrocket". Two years later he married her daughter Irène, the chemist, and thus entered the scientific circle, in the highly stratified society of Paris. Some saw him as a gate-crasher; and he always felt happiest with workers or fishermen.

His research at first brought him some disappointments. In 1931 he published observations which led James Chadwick to the discovery of the neutron and to the Nobel Prize; the next year he narrowly missed the discovery of the positron. But then at last came success: early in 1934 he and his wife published their observation of "artificial radioactivity". That important discovery brought them jointly the 1935 Nobel prize for chemistry and secured for Joliot the first Chair for Nuclear Chemistry, at the Collège de France.

In January 1939 Otto Hahn and Fritz Strassmann published their famous paper which indicated the fission of uranium nuclei struck by neutrons; Irène Joliot-Curie, who had narrowly missed that discovery, "bitterly regretted that she and her husband had not worked together on the problem, for their combined brains might well have solved it". At this point, about a quarter through the book, begins the main story.

Within a few days, Joliot had seen the stupendous implications of that new phenomenon: the possibility of a nuclear chain reaction which could release energy on a large scale. Leo Szilard had been looking for such a reaction since 1934, and Joliot had said in a public lecture in 1935 that "compared with the neutron, the philosophers' stone of the alchemists was a childish fantasy; doubtless people would soon be able to change lead into gold, but this would be an insignificant by-product compared with the enormous quantity of energy that would be released". Now, he felt, it was up to him to create that chain reaction which would present almost unlimited energy to France and hence to the world.



Enrico Fermi in the 1930s

Photo: AIP Neils Bohr Library

First of all he had to make sure that the fission of uranium nuclei released neutrons, as expected. Hans von Halban, son of an Austrian-born professor, was in Paris and keen to join Joliot in tackling that tricky problem, having gained experience with neutrons in Copenhagen (jointly with the reviewer). He in turn recruited Joliot's "little typist", a huge Russian called Lew Kowarski who was determined to become a scientist and did secretarial work to get a foot in the door and to supplement his meagre income from design work in a factory of gas pipes. Trained as a chemical engineer (see my obituary notice of Kowarski in *Nature*) he brought a valuable new way of thinking into the team. Those were the three musketeers who soon agreed that in publishing their joint work they would always share the credit for all ideas, whoever had thought them up.

By the end of February they knew that a chain reaction was not impossible: uranium produced more fresh neutrons than it swallowed. But were there enough of them? Some would escape from the material or be absorbed by its impurities. The team had to know the average number of neutrons released when a single uranium nucleus suffered fission, and by the end of April they thought that number was about 3.5. Today we know that the number is nearer 2.5, but perhaps the error was to the good; it made a chain reaction look easy to achieve and thus encouraged the team to redouble their efforts.

From the beginning they had studied uranium salts dissolved in water, in a tank about 1 m in diameter, with a neutron source at the centre. The neutron distribution in the tank was measured by placing small foils of dysprosium at various distances from the source and, after a standard time, measuring their radio-activity with a Geiger counter; that gave information on the number and the average speed of the neutrons. The water prevented most neutrons from leaving the tank and thus escaping scrutiny; it also served as a 'moderator' which slowed down the neutrons and made them more effective in causing fission. Promising as the results were they came nowhere near a self-sustaining reaction; it was soon clear that much more uranium would be needed.

In May 1939 Joliot approached the Union Minière du Haut-Katanga, a Belgian copper-mining firm which, almost as a sideline, extracted radium from its uranium ore. They had plenty of surplus uranium, and Joliot got them to ship five tons of pure uranium oxide to his Laboratory for Nuclear Synthesis at Ivry (near Paris), with its huge hangar and heavy handling equipment. In July Halban began the tedious neturon density measure-ments on spherical metal containers filled with a black mud, a mixture of uranium oxide and water, up to three atoms of hydrogen to one of uranium; the oxide would not soak up more water. When War broke out in September they had tested



Frédéric Joliot and Irene Curie in 1935

Photo: Radium Institute



Frédéric Joliot, Hans Halban and Lew Kowarski with a geiger counter in 1939

Photo: Radium Institute

spheres up to half a metre in diameter and had clear evidence of a chain reaction that roughly doubled the number of neutrons that came from the source; but it was still far from self-sustaining. Water was not a good moderator; too many neutrons failed to cause fission because they were captured by its protons.

But what other moderator could one use? Carbon, as graphite, was available by the ton, but with its nuclei twelve times heavier than protons, neutrons needed many more collisions to become slow. Would they survive so many collisions without being captured? Much time was lost experimenting with piles of solid carbon dioxide ('dry ice') and industrial graphite; the results were promising but not conclusive. In the USA, similar work was done later and more carefully, convincing Fermi that graphite could be used; about the same time, a wrong result caused the Germans to abandon graphite as a moderator.

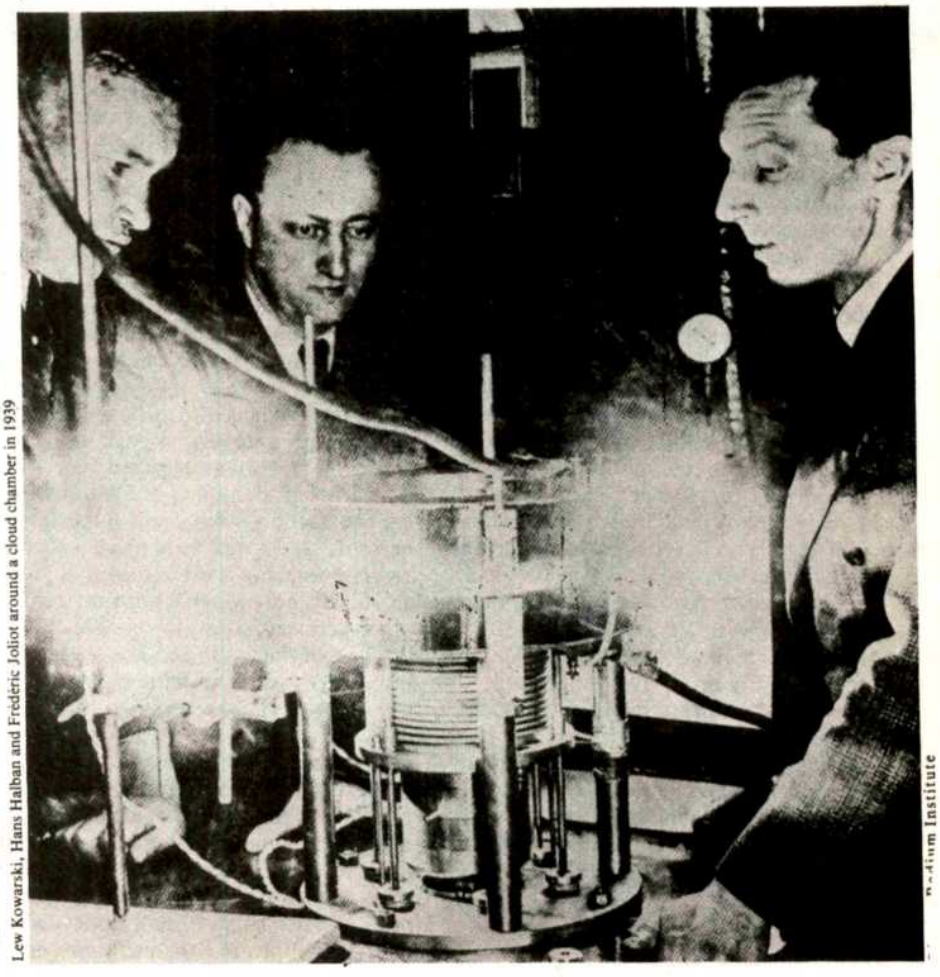
In the meantime Kowarski urged his team to consider heavy water: instead of protons it contains deuterons, only twice their mass, and it hardly absorbs neutrons. But it has to be extracted, at great cost, from ordinary water, of which it forms a minute fraction; in the USA, available only by the tumbler, its use was quickly dismissed. But in Europe the Norwegian firm Norsk Hydro had made almost 200 litres and were ready to increase production. In February, lieutenant Jacques Allier, a former employee of the French bank that controlled Norsk Hydro, went to Norway and persuaded them to hand over their whole stock, free of charge. The Germans were expected to interfere by force, and the entire operation reads like a spy thriller. Until the heavy water arrived in Paris in the middle of March, those two dubious foreigners, Halban and Kowarski, were interned for three weeks on two separate islands!

But in May the German army invaded France. After a brief attempt to set up a new laboratory in Clermont-Ferrand, Joliot decided that the team must split up; he would remain in France while Halban and Kowarski were to resume the work abroad. On June 17 they drove west in their cars, with their families, laboratory notes and the heavy water; in Bordeaux they embarked on a British collier which safely got them to London on June 22, just as France signed an armistice.

The British installed the team and gave them help at the Cavendish Laboratory, Cambridge. An aluminium sphere was constructed and filled with mixtures of uranium oxide and heavy water; it was made to rotate slowly to prevent the oxide settling to the bottom. After five months they had good evidence that a still larger sphere would give a self-sustaining reaction. But Fermi was still sceptical. Moreover, the USA were suspicious of Halban who stressed the French patents and got help from ICI (Imperial Chemical Industries); why help those probable post-war competitors? Negotiations dragged on for nearly three years.

To remove the work from this beleaguered island, a transfer to Canada was prepared; but when it at last took place late in 1942 — just as Fermi won the race for the chain reaction — some of the Cambridge physicists decided to stay with Kowarski, who had refused the inferior position Halban had offered him in Canada. Both teams had to live through another year of slow progress and low morale.

The Quebec Meeting of Churchill and Roosevelt finally enabled Canada to get information and heavy water from the USA. Replacing Halban, (Sir) John Cockcroft took charge of the Canadian team which was now joined by the Cambridge scientists. It took Kowarski less than a year to build a heavy-water reactor,



Lew Kowarski, Hans Halban and Frédéric Joliot around a cloud chamber in 1939

Radium Institute

which began to operate — the first outside the USA — a month after the War came to an end.

Joliot led a double life during the occupation. He kept his Paris laboratory going, but subtly sabotaged any research which Germany tried to conduct there; and he worked in the Resistance and came to admire the communists for their courage, idealism and competence. When France was liberated he was tireless in reviving fission research, amidst political strife and a ruined economy. Equipment was requisitioned or home-made; young people, attracted by the glamour of nuclear energy, were trained. Before Christmas 1948 the first reactor in France began to operate a heavy-water reactor, again built by Kowarski.

After that, the power of the scientists began to decline as the engineers, administrators and politicians took over. The declaration that France would never build nuclear weapons was gradually forgotten. Joliot's communist stance caused increasing problems, and after a rally in April 1950 when he declared that France would never make war on the Soviet Union he was removed from his post as head of the Commissariat à l'Energie Atomique. Large reactors were built, optimised for the production of plutonium, and the first French Bomb was exploded over the Sahara in 1960.

Joliot is clearly the hero of the book, a scientist who combined hard logic with lively imagination, and a persuasive personality with considerable skill as organiser. In his bid for nuclear power he was up against Fermi, probably the greatest all-round physicist of our time, as experimenter and theoretician alike, while France was short on theory. But Fermi was cautious and a foreigner in the USA while Joliot was full of fire and had the support of his country and his government. The author quotes Lord Blackett for saying "had the war not intervened, the world's first self-sustaining reaction would have been achieved in France".

This is a very well researched book, with its 12 pages of bibliography, 35 of footnotes, and a good index. But in spite of that scholarship the book is eminently readable and contains deft pen portraits of its many characters. It is written with a quiet dignity that yet does justice to the dramatic events with which it deals. Spencer Weart focusses on the French story but gives fair attention to efforts made elsewhere. Scientific explanations are kept fairly brief; much more space is given to the delicate interplay of social, economic and political forces; of great families and great schools; of scientists and administrators. Whichever way you look at it, this is a valuable and fascinating book. □

Otto R. Frisch was Professor Emeritus at Trinity College, Cambridge, UK. In Copenhagen he had contributed to the discovery of nuclear fission early in 1939. Professor Frisch died on 22 September, 1979.

Curiosity of a scientist

Hermann Bondi

Disturbing the Universe. By Freeman Dyson. Pp. 283. (Harper and Row: New York and London, 1979.) $12.95; £6.50.

OCCASIONALLY I feel unkind towards my many friends and colleagues in the education industry by pointing out that every small child makes a nuisance of itself by constantly asking "Why?", that society has developed a marvellous defence mechanism called education, and that this is so effective that it stops almost everyone from keeping on with this questioning. The few failures of this cure are, I claim, called scientists.

Curiosity must indeed be the mainspring of scientific endeavour. The constant nagging feeling of *wanting* to know something or other that is well hidden is indeed a powerful drive. To find out what a particular problem really is like and has as its solution, to worry the problem like a dog worries a bone, to gain insight by following to their end innumerable false turns, to see the whole confusing jumble eventually form a surprising and unexplored pattern, that is the real compulsive urge and joy of a scientist's labours.

To an extent that varies markedly between individuals there is associated with this an urge to communicate, an urge to teach, an urge to show to others the lovely pattern one has seen (whether one has found them or made them is a moot point) in one's own sandbox. Thus, research and teaching are naturally linked, but the link may take many different forms. To interest suitably qualified colleagues or research students in one's own researches is natural enough, but to watch a research student learn by making his own mistakes, perhaps messing up a lovely corner of one's sandbox is more of an acquired taste needing much self-discipline, but with its own rich satisfaction when one's pupil finally soars off under his own power. Undergraduate teaching is often seen as a chore, but only by those who have not got the urge to get across the beauty one sees in the structure of one's subject, the pleasure of conveying the personal pattern that one has seen (or made) in a well known part of the field.

Thus, there is substance to the notion of the normal academic career where one's researches (the value of which can be judged reasonably well) lead to posts in which teaching (the quality of which it is much harder for a university to assess) forms a major part of one's duties, along with continuation of one's research. However, justification for the curious habit of academic tenure (other than the mental comfort of the holder) can surely only be found if it enables the incumbent to follow daringly different unconventional and potentially fruitful pursuits on which he would otherwise not have embarked. Unhappily it is only a minute minority who so use their freedom. The vast majority of tenured academics, even of full professors who have nowhere further to rise, turn their academic positions or chairs, not so much into comfortable beds, as into narrow grooves of dried-up specialism often made most uncomfortable by making an 80 hour week out of administrative tasks that would take a competent bureaucrat half of an afternoon. Rare indeed is the person who really uses the freedom given by academic tenure to enrich the world and to enjoy himself by working hard and effectively at each in turn of the wide variety of pursuits he finds intriguing. Pre-eminently one such is Freeman Dyson, and his book is an exciting journey through the broad landscape of his experiences and thoughts.

Perhaps this is the point at which I should interpose the story of my period of friendship with the author which illustrates some of the bits he so oddly left out of the book. He does not mention his extraordinary brilliance at school at Winchester and as undergraduate at Trinity College, Cambridge, in the bumper class that had in it James Lighthill and Freeman Dyson, a record pair not equalled before or since. After the painful interlude of Bomber Command (fully described in the volume) he returned to Trinity as a young Fellow a few years my junior. An inveterate talker like myself (I recall his interrupting me at High Table dinner with "Not another word now. We are still on the soup while the other are finishing their desserts") he clearly had a most enjoyable year, but found that Cambridge, with its then rigid barrier between Physics and Mathematics was not a good place to learn and work in the lively field of theoretical quantum physics he wished to pursue. Hence his move to Cornell and Hans Bethe, where his book restarts. Why this odd omission of Cambridge?

He relates how he learned, travelled, and did brilliantly, none of which surprised me. But I was astonished and a little worried when he accepted a permanent position at the Institute of Advanced Studies in Princeton. That such an able and enterprising young man should take a post from which there was no escape upwards in academic life, a post that would hang round his neck for nearly 40 years, that would prevent him from teaching anyone but a few highly selected graduate students, that would isolate him in a closed esoteric community, all this saddened me greatly. How wrong I was to worry is so beautifully shown in his book. To him the Institute post was simply a springboard for pursuing

The Biology of the Lymphokines

edited by Stanley Cohen, Edgar Pick and Joost J. Oppenheim

December 1979/January 1980, 656pp., £18.50/$42.50 0.12.178250.6

The Biology of the Lymphokines is the first volume devoted entirely to the subject of lymphokine biology. The leading investigators in this burgeoning field contribute up-to-date reviews on major areas such as the cellular origin, biochemical characteristics, mechanism of action, biological relevance, pharmacology, and clinical application of lymphokines. A well-balanced view is provided of the multiple involvement of lymphokines in immunological as well as non-immunological events. The synthetic treatment of the subject makes this book essential for immunologists, cell biologists, biochemists, microbiologists, haematologists, pathologists, and medical researchers generally.

Sexual Selection and Reproductive Competition in Insects

edited by Murray S. Blum and Nancy A. Blum

September 1979, 476pp., £14.95/$23.00 0.12.108750.6

Contents include: D. Otte: Historical development of sexual selection theory. **G. Borgia:** Sexual selection and the evolution of mating systems. **R. Thornhill:** Male and female sexual selection and the evolution of mating strategies in insects. **G. A. Parker:** Sexual selection and sexual conflict. **W. D. Hamilton:** Wingless and fighting males in fig wasps and other insects. **D. K. McAlpine:** Agonistic behaviour in *Achias australis* (Diptera, Platystomatidae) and the significance of eyestalks. **W. G. Eberhard:** The function of horns in *Podischnus agenor* (Dynastinae) and other beetles. **D. Otte and K. Stayman:** Beetle horns: some patterns in functional morphology. **J. E. Lloyd:** Sexual selection in luminescent beetles. **W. Cade:** The evolution of alternative male reproductive strategies in field crickets.

Frontiers in Physicochemical Biology

Proceedings of an International Symposium held at the Institut de Biologie Physico-Chemique, Foundation Edmond de Rothschild, in May 1977

edited by Bernard Pullman

March 1979, 528pp., £29.25/$45.00 0.12.566960.7

The twenty-one papers assembled in this volume represent a unique, overall survey of the important developments in nucleic acids and protein interactions, genetic regulation, molecular biology of oxygen and haemoglobin, and the bioenergetics of organised systems from a biophysical and biochemical perspective. They have been written by the world's most eminent specialist in the field. The book is divided into four main parts: The physical chemistry of fundamental biomolecules; The physiochemical aspects of the mechanisms of genetic expression; Biochemistry of oxygen and haemoglobin; Study of organised systems.

Cell Biology and Immunology of Leukocyte Function

Proceedings of the Twelfth International Leukocyte Culture Conference held at the Ben Gurion University of the Negev, Beer Sheva, Israel, June 25-30, 1970.

edited by Michael R. Quastel

August 1979, 1002pp., £29.40/$45.00 0.12.569650.7

The book opens with a group of papers on the mechanism of lymphocyte activation, a subject of perennial interest, and the main theme running through all previous volumes of the series. Subsequent sections deal with lymphocyte differentiation, the genetic control and intracellular interactions involved in leukocyte function, parameters of the immune response under *in vitro* conditions, the interactions of viruses and lymphocytes, and the application of leukocyte culture to problems of clinical medicine. The final part of the book involves a new subject for this series, the study of radiation effects and leukocyte malignancy.

Marine Pollution

Functional Responses

edited by Winona B. Vernberg, Frederick P. Thurberg, Anthony Calabrese and F. John Vernberg.

September 1979, 464pp., £15.00/$23.00 0.12.718260.8

From the preface: The papers in this volume were presented at a symposium entitled "Pollution and Physiology of Marine Organisms" held at Hobcaw Barony, Georgetown, South Carolina in November 1977 . . . There has been a veritable explosion in interest in this subject in the past few years, and we are grateful to Carl Sindermann for his thought-provoking and incisive overview of our present level of knowledge as well as charting where our future needs lie. It is clear that we have long passed the point where death is the only criterion for determining whether a substance is harmful or not and the large number of papers on sublethal effects in this volume attest to this present trend in physiological pollution studies.

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Ultrastructure of the Rust Fungi

Larry J. Littlefield and Michele C. Heath
October 1979, 296pp., £24.80/$38.00 0.12.452650.0

Contents: Morphology and ontogeny of sori and spores: Basidiospores. Pycnia and Pycniospores. The Dikaryotization Process. Aecia and Aeciospores. Uredia and Urediospores. Telia and Teliospores. Overview. **Infection of the susceptible host:** Directional Growth of Urediospore Germ Tubes. Induction of Appressoria. Development of Infection Structures. **Vegetative growth in the susceptible host:** Intercellular Hyphae. Intracellular Structures. **Nuclei:** Interphase Nuclei. Nuclear Division. **Incompatible plant-rust associations:** "Natural" Resistance. Resistance Elicited by Systemic Fungicides. **Parasites of rust fungi:** Fungi. Viruses.

Versatility of Proteins

Proceedings of an International Symposium on Proteins held on March 6-8 in Taipei.

edited by Choh Hao Li
March 1979, 408pp., £14.30/$22.00 0.12.447750.X

Contents include: K. A. Walsh et al: Strategies of amino acid sequence analysis. **T. Wieland:** Solution vs. solid-phase methods in peptide chemistry. **D. Yamashiro and J. Blake:** Improved procedures for the solid-phase synthesis and purification of peptides. **J. T. Yang and C.-S. C. Wu:** Surfactant-induced conformation of some oligopeptides, polypeptides, and proteins: helix- and β-forming potential of amino acid sequence in proteinaceous environment. **H. A. Scheraga:** An approximate model for protein folding: experimental and theoretical aspects. **E. H. Fischer et al:** Evolutionary aspects of the structure and regulation of phosphorylase kinase.

The Role of Insectivorous Birds in Forest Ecosystems

Proceedings of a symposium, The Role of Insectivorous Birds in Forest Ecosystems, held on July 13-14, 1978, in Nacogdoches, Texas, sponsored by the School of Forestry, Stephen F. Austin State University, and the USDA Forest Service, Southern Forest Experiment Station, Wildlife Habitat and Silviculture Laboratory.

edited by James G. Dickson, Richard N. Conner, Robert R. Fleet, Jerome A. Jackson and James C. Kroll.
October 1979, 400pp., £15.60/$24.00 0.12.215350.2

Contents include: Censusing Birds: L. J. Folse Jr.: Analysis of community census data: a multivariate approach. **W. M. Shields:** Avian census techniques: an analytical review. **Sampling Prey Populations: R. Coulson et al:** Sampling considerations for evaluating the effects of mortality agents on bark beetles. **J. A. Jackson:** Tree surfaces as foraging substrates for insectivorous birds. **Foraging Strategies: R. N. Conner:** Seasonal changes in woodpecker foraging methods: strategies for winter survival.

The Study of Prosimian Behavior

edited by G. A. Doyle and R. D. Martin
July 1979, 704pp., £32.15/$49.50 0.12.222150.8

This book gives perhaps the first comprehensive, state-of-the-field picture of research on prosimian behaviour. With the growth of research in this area, a book on the subject is now possible. The contributors to *The Study of Prosimian Behavior* report experimental laboratory studies, studies of normal behaviour in semi-natural settings, and studies in the natural habitat by means of such sophisticated instruments as infrared devices and radio-tracking equipment. Topics covered include: reproduction and maternal behaviour; learning and intelligence; diet; locomotion; ranging behaviour; vision and olfaction; classification; phylogeny.

Cell-Associated Water

A workshop — organised in connection with the First International Congress on Cell Biology, Boston, September, 1976.

edited by W. Drost-Hansen and James S. Clegg
July 1979, 450pp., £14.30/$22.00 0.12.222250.4

Contents include: J. E. Morel and M. P. Gingold: Does water play a role in the stability of the myofilament lattice and other filament arrays? **P. M. Wiggins:** Metabolic control of the properties of intracellular water as a universal driving force for active transport. **R. M. Hurtado and W. Drost-Hansen:** Ionic selectivities of vicinal water in the pores of a silica gel. **F. M. Etzler and W. Drost-Hansen:** A role for water in biological rate processes. **C. F. Hazlewood:** A view of the significance and understanding of the physical properties of cell-associated water.

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Antibody Production in Man:

In Vitro Synthesis and Clinical Implications

Anthony S. Fauci and Rudy E. Ballieux

September 1979, 408pp., £11.80/$18.00 0.12.249950.6

From the preface: On March 15-18, 1978, the Catharijne Conference was held in Utrecht, Holland . . . The workshop was divided into four major sessions preceded by an introductory lecture by Dr. Max D. Cooper entitled "Consideration of Antibody Deficiency Diseases in Relation to Normal B Cell Development". The actual workshop sessions were concerned with mitogen induced B cell activation, antigen induced B cell activation, subpopulations of lymphocytes involved in the regulation of *in vitro* B cell function, and clinical implications in the study of *in vitro* B cell function.

Pine Barrens: Ecosystem and Landscape

edited by Richard T. T. Forman

November/December 1979, 624pp., £25.65/$39.50 0.12.236450.0

Contents include: People: P. O. Wacker: Human exploitation of the New Jersey Pine Barrens before 1900. **J. E. Applegate et al:** Plant and animal products of the Pine Barrens. **Geology and Soils: E. C. Rhodehamel:** Geology of the Pine Barrens of New Jersey. **Climate, Water and Aquatic Ecosystems: A. V. Havens:** Climate and microclimate of the New Jersey Pine Barrens. **E. C. Rhodehamel:** Hydrology of the New Jersey Pine Barrens. **Vegetation Patterns: C. J. Heusser:** Vegetational history of the Pine Barrens. **Plants: F. T. Ledig and S. Little:** Pitch pine *(Pinus rigida Mill):* ecology, physiology and genetics. **Animals and Animal Communities: L. J. Wolgast:** Mammals of the New Jersey Pine Barrens. **C. F. Leck:** Birds of the Pine Barrens. **R. Conant:** A zoogeographical review of the amphibians and reptiles of southern New Jersey with emphasis on the Pine Barrens. **R. W. Hastings:** Fish of the Pine Barrens. **H. P. Boyd and P. E. Marucci:** Arthropods of the Pine Barrens. **Conclusion.**

Chemical Compounds in the Atmosphere

T. E. Graedel

September 1979, 456pp., £19.15/$29.50 0.12.294480.1

A unique, practical encyclopedic reference work for atmospheric scientists, chemists, chemical engineers, and workers in the environmental sciences, this is the first comprehensive assemblage of information on atmospheric compounds. It provides data on the sources, ambient concentrations, reactions, products, and atmospheric lifetimes of more than 1600 compounds emitted into, or present in, the Earth's atmosphere. Information has been gathered and synthesized from over 800 sources in fields ranging from forestry to physics. Three different cross-reference tables make the material conveniently available to users with a variety of interests.

Viruses and Environment

Proceedings of the Third International Conference on Comparative Virology, held at Mont Gabriel, Quebec, Canada, in May 1977.

edited by Edouard Kurstak and Karl Maramorosch

July 1979, 696pp., £20.80/$32.00 0.12.429766.8

Viruses and Environment presents the current trends prevailing in virus research, and the deep implications and the role of viruses in biological and environmental studies. The scope and objectives of this work encompass the impact of viruses on the environment, the latest findings concerning persistent virus infections of man, vertebrates, invertebrates, and plants, as well as a discussion of the smallest disease agents, the viroids. Among the timely areas presented are the discussion of the precautions to be taken in the use of live viruses either as vaccines or biological control agents and in genetic engineering.

Physiology of Woody Plants

Paul J. Kramer and Theodore T. Kozlowski

September 1979, 832pp., £22.75/$35.00 0.12.425050.5

Both a basic text and a practical reference, this comprehensive, authoritative synthesis brings together the current state of knowledge on how woody plants grow. It describes in detail the physiological processes and structural features involved in growth as well as the internal, environmental, and cultural factors influencing growth. The book differs from most textbooks on plant physiology in that strong emphasis is placed on tree structure and on the interactions of trees and stands of trees with their environment. It is well documented with more than 2000 references from the world literature.

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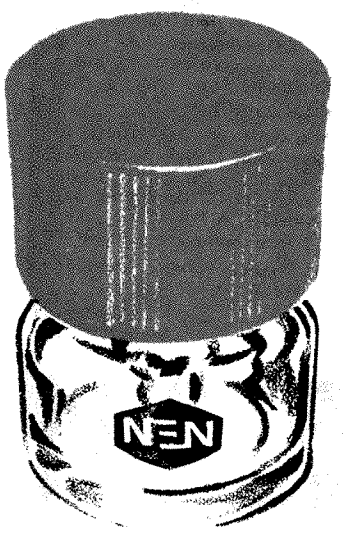
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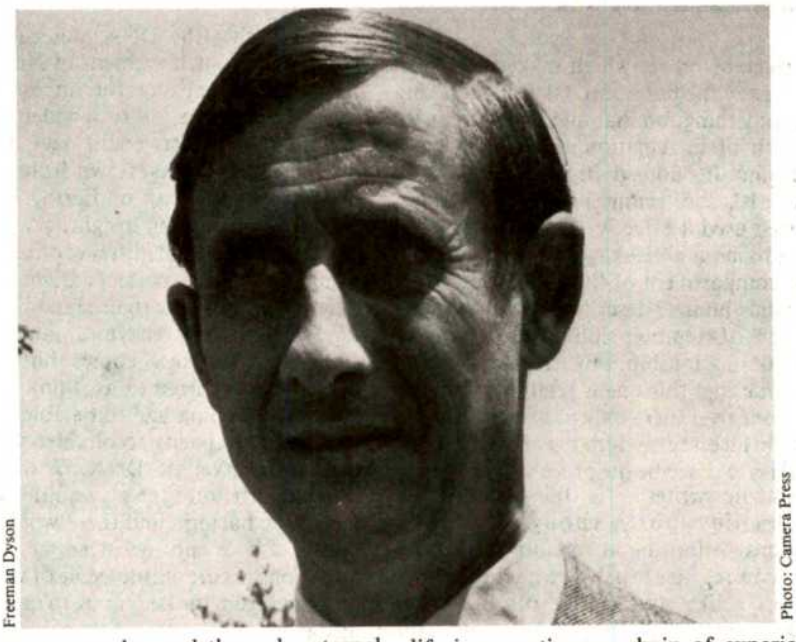
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his foibles, and for one such as Dyson each foible is both what would be the adventure of a lifetime for others and a notable contribution to mankind's storehouse of knowledge and experience.

Two of his particular characteristics are to be totally fascinated by space, and to be deeply interested in nuclear power. He describes with such evident joy his cooperation with a brilliant group of people to design the 'inherently safe' Triga nuclear research reactor. In very little time the endeavour was most successful, and dozens of these reactors were sold. Not long afterwards Dyson was involved with the splendidly conceived Orion project which aimed to use nuclear power to make space travel cheap and fast. In the end, this endeavour was doomed through external developments. He describes his involvement with molecular biology more sketchily but the depth of his understanding is evident. Politics fascinated him, not the strident shouting of electioneering that is so liable to expand minor differences of judgment on non-central issues into great philosophical divisions, but the patient work of exploring how the most deeply held and widely differing convictions can be reconciled in modest and valuable programmes of action. His work in the Arms Control and Disarmament Agency and his efforts in the Princeton Citizen's Committee to advise on whether recombinant DNA researches should be permitted are all lovingly related in a most human and readable manner. Much earlier, in the Oppenheimer tragedy, he seems to have been only a spectator, naturally an immensely concerned spectator, but one with much understanding of how some actors came to what he regards as utterly wrong choices. This was his blooding in politics, and if it left him wounded, he was keen to point out the total absence of scars.

But perhaps the most thrilling part of the book deals with his deep speculations on mankind's future in space as he sees it. I happen to be more of a doubter in this field, but I too was carried along by the sweep and logic of his vision, by his imaginative application of knowledge, by his sheer enthusiasm. I tend to think that the solution of our problems on the Earth must be found on the Earth, but his comparison with older efforts of settling new lands is certainly intriguing and well argued, his view of the need for adventure is stimulating, and the whole section is not just very readable, but truly exciting and thought provoking.

I am less enthusiastic about the pieces of Dyson's personal history in the book. They certainly are well chosen fragments, but they are only fragments. One's personal life is a continuous chain of experiences and impressions, each section being properly intelligible only in the light of its predecessors. His episodes, though, make excellent reading matter. He is also curiously reticent about his scientific work since his appointment to the Institute.



Freeman Dyson

Photo: Camera Press

But to come back to the mainstream of his book, I was especially intrigued by his fascination with engineering, a fascination I, as another theoretical physicist, share with him, though he came to it earlier in life than I did. But where he and I differ is in our attitudes to bureaucracy. It is natural for a scientist to feel antipathetic to big organisations. The brilliant ideas, the outstanding contributions we most admire and indeed venerate, are necessarily the product of one person's mind, or very occasionally of two or three closely collaborating minds. Ten thousand mediocrities working for ten thousand years could not have duplicated Einstein's ideas or those of Charles Darwin. Exceptional quality of mind is needed in our fields and cannot be replaced by quantity. But great engineering projects like Apollo, or the Forth Bridge, or Concorde, are created, and can only be created, by vast impersonal organisations responding only slowly and gently to any

ELLIS HORWOOD

Publishers Chichester

CELL POPULATIONS

Editor: **E. Reid,** *Director, Wolfson Bioanalytical Centre, University of Surrey*

This desk guide to new and established methods of isolating cell populations from tissues or other heterogeneous biological sources surveys techniques for obtaining homogeneous samples of healthy cells from mammalian tissues, and from protozoan and other free-living cells. It also offers guidance on the choice of the most suitable methods. The range of techniques covered includes sedimentation, isopynic banding, elutriation, partition between two aqueous phases, and free-flow electrophoresis. It will serve as a research and laboratory reference book, or as a teaching aid, providing also adequate background knowledge for non-specialists. It is an authoritative coverage of the field by an international team of experts, first presented at a Subcellular Methodology Forum held at the University of Surrey in 1978. There is no such wide-ranging survey of its size known to be available.

Contributors include: W.S. Bont, N. Crawford, M.J. Crumpton, W.H. Evans, R.J. Hay, H.G. Heidrich, G. Johansson, D.L. Knook, H.A. Krebs, S.M. Lanham, J.P. Luzio, H. Pertoft, G. Raydt, I. Schulz, P.O. Seglen, and Th. J.C. van Berkel.

Series: *Methodological Surveys (Series B: Biochemistry) Vol. 8*

October 1979 252 pages
085312 120 6 $45.15/£18.50

PLANT ORGANELLES

Editor: **E. Reid,** *Director, Wolfson Bioanalytical Centre, University of Surrey*

This comprehensive and critical survey of known methods of the isolation of organelles and membrane material from plant tissue also emphasizes the advances in the field.

The book first considers methods for isolating bioenergetic organelles, and then presents current views on vacuoles and microbodies. There is consideration of membranous components and nuclei, and of constituents such as glucan synthetases and phytochrome, with attention to lectins, auxin binding and glycosyl transferases. The critical collation of current thinking on markers for characterizing subcellular fractions concludes this outcome of a Subcellular Methodology Forum held at the University of Surrey in 1978.

Contributors include: H. Beevers, D. J. Bowles, R. Douce, J. L. Hall, R. Hertel, C. Larsson, R. A. Leigh, J. M. Lord, G. Maclachlan, A. E. C. MacRobbie, D. Marmé, D. J. Morré, C. A. Price, P. H. Quail, P. M. Ray, S. P. Robinson and E. L. Vigil.

Series: *Methodological Surveys (Series B: Biochemistry) Vol. 9*

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change in direction, whether the command comes from a genius or from a mediocrity. They can be accelerated slightly be leaders of great drive, and lose momentum without leadership. But invariably they grind on remorselessly, very inefficiently, but necessarily successfully, for only mass murder could stop them in their tracks.

It needs a certain type of sophistication to derive job satisfaction from the awareness that, without one's sustained efforts over a year, the organisation would be moving in a slightly different direction, and would not have got quite so far. Such a form of sophistication Dyson does not have. To him only work in a small group is fun. This attitude separates him from much modern engineering and from much that is involved in the public service. He is thus a supporter of small bands of pioneers rather than of large project management teams. It is a great loss that our necessarily large bureaucracies are unable to attract such talent.

I am sure I have said enough to make it clear that I thoroughly enjoyed reading this book, that it gave me a lot to think about, and that I can recommend it heartily to all. □

Sir Hermann Bondi is Chief Scientist at Her Majesty's Government's Department of Energy.

A man who thinks otherwise

Eric Ashby

The Medusa and the Snail: More Notes of a Biology Watcher. By Lewis Thomas. Pp. 175. (Viking: New York, 1979.) $8.95. (A UK edition will be published after May next year by Allen Lane (London).)

IN far off days, when the academic profession was a revered élite, the Germans defined a professor as "a man who thinks otherwise". In these days, when knowledge is fragmented and the academic profession unionised, otherwise-thinking is rare, even among professors. Hence the significance of this little book of essays, for Lewis Thomas is a virtuoso in thinking otherwise.

At first glance the book seems to be lightweight, no more than a capricious assortment of fancies about ponds in Manhattan, cloning of human beings, committees, punctuation, disease, death, warts; all gently mischievous, witty, conversational in style, the work of a latter day Montaigne or Lamb. Those who have read Thomas' other essays (*The Lives of a Cell: Notes of a Biology Watcher;* Viking: New York; Garnstone: Petworth, UK; for review, see *Nature*, **258**, 24; 1975), know how misleading such a first glance would be. In common with another Lewis, Lewis Carroll, he has an extraordinary intellectual exuberance. I have not read any of his papers on pathology (Thomas is a distinguished pathologist) but I guess they are as unlike *The Medusa and the Snail* as Lewis Carroll's *Euclid and his Modern Rivals*, published just 100 years ago, is unlike *The Hunting of the Snark*.

Carroll wrote for children. Thomas writes for grown-ups who still retain a child's capacity for wonder. Apart from his mastery of prose, which makes me green with envy, he has a rare talent for picking up something familiar and suddenly, with a flash of imagination, giving it a fresh meaning. It's done deftly, like a conjuring trick. It's the technique of a poet. Edith Sitwell used it effectively; she described it once to me as a "leakage of thought" from one compartment of the mind to another. Thomas himself describes it: "finding the marks of remote similarity, points of distant relationship, tiny irregularities that indicate that this one is really the same as that one over there only more important". And in an essay on thinking about thinking he tries to describe the process. The human head, he writes, "is filled alive with molecules of thought called notions. . .flowing at random from place to place, bumping against each other. . .like the paths of Brownian movement". The mind is heated a little, the movement speeds up, there are more encounters, some of the notions dock and lock (notice the imagery of space-travel), aggregates are formed, they act like "purposeful organisms, hunting for new things to fit with. . .turning things over, catching at everything" — and a harmonious idea is generated, with the elegant geometry of a Brandenburg concerto. At that point Thomas' mind takes another leap: why not begin with music "and see what this can tell us about the sensation of thinking".

So far so good: the book is delightful entertainment. Is it more than that? I believe it is. It is a piece of intellectual autobiography. Its theme is the impact of Nature on Lewis Thomas. By what I believe is a valid extrapolation, it speaks for thousands of other less articulate biologists and naturalists; and this is why it is more than entertainment. Thomas visits the Tucson Zoo and watches beavers and otters at play in glass-walled pools. For a few minutes he sheds his professional reductionist shell and becomes, as he puts it, "transfixed. . .there was only one sensation in my head: pure elation mixed with amazement at such perfection". The beavers and otters had done something to him. "I learned nothing new about them. Only about me, and I suspect also about you, maybe about human beings at large: we are endowed with genes which code out our reaction to beavers and otters, *maybe* [my italics] *our reaction to each other as well*".

Speculation, bordering on the turbulent territory of sociobiology? Perhaps it is, but there is no dogmatic assertion in Thomas' reflections. He offers you a subjective experience, take it or leave it. I would be surprised if many biologists reject the *credo* which is the main motif in these essays. The *credo* rests on wonder at two principles Thomas finds all around him. One is uniqueness; the other is symbiosis. Here are a few examples of the impact they make on him.

To say that the DNA molecule is the greatest single achievement of Nature is a commonlace opening for an essay. But then Thomas goes on to wonder whether molecular biologists could have achieved this, if they had been flown from another planet to start life off on Earth. Suddenly comes the refreshing insight: "We would have made one fatal mistake: our molecule would have been perfect. Given enough time, we would have figured out how to do this, nucleotides, enzymes, and all, to make flawless, exact, copies, but it would never have occurred to us, thinking as we do, that the thing had to be able to make errors. The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music. . .it is no accident at all that mutations occur; the molecule of DNA was ordained from the beginning to make small mistakes".

This is the source of the uniqueness of living things, of the idea of selfhood. In the *Testament of Beauty* Robert Bridges came to a similar conclusion along a different path. Thomas takes the scientist's path. He cites the self-labels of beans — the glycoproteins which may negotiate the intimate concordat between bean and nitrogen-fixing bactera; he cites the capacity of fish and mice to tell each other apart as individuals by a sense of smell; and he concludes that nucleated cells detached from the parent organism seem to be the only living units without a "sense of privacy". It is no wonder he has deep reservations about cloning human beings. Of course, like any other professional biologist, he doesn't think it is likely to happen; but suppose it did: think what this would do to filial affection and paternal care; think of the terrifying prospect of raising from infancy and teaching language and discipline to a creature whose genetic constitution is exactly the same as your own. In any case, as Thomas explains, it couldn't happen because the selfhood of human beings depends not only on their genes but also on their environment, including their personal relations with other human beings, and it would be

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impossible to homogenise this environment. And it's as well it couldn't happen, for the only hope for evolution is for the race to have options to be matched against diversity. There's a deep wisdom in the phrase "trial and error"; we don't say (Thomas reminds us) "trial and rightness" or "trial and triumph".

One theme in these essays is to praise uniqueness. The other is to praise symbiosis and cooperation. Uniqueness does not spell solitude. The individuals cannot express their selfhood except in some sort of communication with other individuals. The committee, the quorum, the consortium, the working party: these are not specifically human devices for getting things done — they were invented long before by all manner of lowly organisms. Collaboration between organisms of the same kind and symbiosis between organisms of different kinds are so widespread that it leads Thomas to say that "the committee is the basis of terrestrial life". This carries him, at a safe distance from sociobiological analogies, to a discussion of the behaviour of committees of human beings. He has some penetrating things to say about the way we behave on committees. Each member, being unique, "is necessarily an actor, uncontrollably acting out the part of himself, reading the lines that identify him, asserting his identity". This is an obstacle to the efficacy of human committees, not shared by consortia of ants or termites. Each member's function, Thomas says, is to talk, "and while other people were talking the individual member was busy figuring out what he ought to say next in order to shore up his own original position". With more confidence than I myself have, Thomas advocates the Delphi technique invented by the Rand Corporation. His support for it rests on good grounds, namely that the members don't meet; instead they fill out a succession of forms, on which their views are (so it is hoped) modified by the written views of other members of the group. Hence the group members are not in competition to uphold their selfhood; instead they have time to think. Well, perhaps so. But this is surely to overlook another principle governing Nature, namely that decisions, whether consciously made by human beings or unconsciously reached by the mechanism of evolution, are the results of conflicts; and to hold a conflict one has to have an arena, even if it is a committee room.

I have space only to mention a few other flashes of illumination in the book. Thomas knows a lot about linguistics and he points out the difficulties of discussing the meanings of words when the tool for research is words themselves. There is a sort of Heisenberg effect which may distort the argument. Over the applications of science to medicine he is an unashamed optimist. He deplores the American obsession with health: "Get a check-up. Go on a diet. Meditate. Jog". Jogging particularly riles him: "swarms of people, out onto the streets each day in underpants, moving in a stolid sort of rapid trudge, hoping by this to stay alive". Why have people lost confidence in the human body, which has survived plague, starvation, pathogens, and is now better protected from these than ever before in all history? Thomas attributes this obsession in part to the ministrations of an immensely costly health care service.

The book, as I said, is about its author. The scientific content is merely the fuel which ignites his imagination. The personality that emerges is not entirely consistent nor is the argument always entirely logical. Thomas himself will, I think, welcome this comment, for in an essay about schizophrenics he protests that far from being a dreadful thing to have more than one self, it can (if the selves do not crowd into the body at the same time) be a rich experience; and indeed Thomas confesses to being several selves, so he would not wish to be thought to be consistent. And as for logic, Freud had the answer to that one: "Only in logic are contradictions unable to exist; in feelings they quite happily continue alongside one another". □



Lewis Thomas

Photo: Helen Marcus

Lord Ashby is Chancellor of Queen's University, Belfast, and a Fellow of Clare College, Cambridge, UK.

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Florey's creative power

E.P. Abraham

Howard Florey: The Making of a Great Scientist. By Gwyn Macfarlane. Pp.396. (Oxford University Press: Oxford and New York, 1979.) £7.95.

THIS book is an authoritative biography of Lord Florey in his early and middle life. It is a tribute to a man who had a central role in the story of penicillin and became one of the great figures in the history of medical science. It is also a product of the persistence of Gwyn Macfarlane, who was told by publishers that an unknown author could not expect to see his biography of a scientist in print unless his subject was already famous in the public mind; and that although Fleming was a household name, Florey was not. Why this was so and how a myth was created are finally discussed in detail. A major theme of the book, however, as its sub-title implies, is the development of Howard Florey as a scientist and the factors that contributed to his success. "To be a great scientist", the author states, "one must (among other things) be a pioneer, a discoverer, a dedicated research worker, and one must be successful"; and he sets out to show how Florey came to fall within this definition.

In his assessment of Florey's character the author, unlike earlier biographers, has quoted from letters that Florey wrote from England and America to Ethel Hayter Reed, a fellow medical student in Adelaide, with whom he thought he had fallen in love. They were referred to later by Ethel as love letters, but *eros* scarcely appears in them although *agape* is sometimes seen. Some of the extracts from this correspondence, between two young people who were fourteen thousand miles apart, seem strangely cautious and business-like. But even those who know the outcome may have a sense of drama and begin to wonder whether marriage will eventually result or not, as problems, difficulties and requirements are laid on the table.

In the event, they married on 19 October 1926 in London a few weeks after Ethel's arrival from Adelaide. The marriage began badly and it was immediately evident that they were unlikely to be perfect partners. She was an attractive woman with intelligence and charm who could write to him on the boat "Do you realise that I shall arrive in England on your birthday: I shall wrap myself up in tissue paper tied with pink ribbon . . . will you accept the present or throw it into the sea?". Yet she was strong willed and difficult to live with and she had written of marriage as an ordeal. He, like many other young men, had ambition, impatience, idealism and private worries about his capability. They were both unduly concerned by thoughts of a lack of money and, with her, fear of poverty seemed to become almost an obsession. Neither had formed positive views of the nature of their relationship. While he had disclaimed any wish to impede her professional life or press her into domesticity, he had also written: "Married women messing about in labs always appear to be slightly pathological to me".

The marriage was not devoid of affection and there were intervals of contentment which coincided with the birth of two children. But it is clear that he did not find in her the ideal companion of his imagination and that some of her own premarital forebodings became realities. He was exasperated by her increasing deafness; yet other marriages have been happy in the face of greater impediments and the reader is left to wonder whether deeper problems were unresolved.

No general principles to guide the young who aspire to become great scientists are likely to emerge from this topic in the letters, for creative power seems to have been unleashed in some men and stifled in others by emotional frustration. There can be no doubt that Florey's epistolatory attachment to Ethel gave him great comfort during his early years in England, when feelings of loneliness sometimes became acute, and that it removed temptation to indulge in diversions which could have interfered with his great enthusiasm for research. But the relevance of his subsequent dissappointment to his great achievements is a matter for speculation — a process of which Florey himself was not much enamoured.

The Florey letters, however, also provide a diary of his early years in Oxford, where he obtained a first class in the Honour School of Physiology, and then in Cambridge, America and London. They describe the experiments he had done and intended to do with laboratory animals on the capillaries, the lymphatics, the omentum and mucus secretion. They tell of his emotional response to music and to the architectural achievements of past centuries and give the critical views of a young Australian on the English society of the day. They contain accounts of his meetings with a number of the great or then powerful figures in preclinical medicine, including Charles Sherrington, A.R. Dean, Edward Mellanby and J.B.S. Haldane. And they show that Florey's poor view of the intellectual content of much clinical teaching and of the quality of clinical research had been formed by the time he had qualified in medicine at Adelaide. All this Gwyn Macfarlane brings together in an admirable manner.

It was Sherrington who suggested to Florey that the time had come for an approach to pathology by a physiologist. Sherrington, Dean and later Mellanby were much impressed by his ability and energy in research and their support helped him to obtain positions at Cambridge and the London Hospital and then Chairs at Sheffield and Oxford. The Professorial appointments were made in the face of powerful opposition, for Florey was not regarded as a pathologist by the morbid anatomists who dominated the profession; Macfarlane tells the well-authenticated story of Mellanby arriving late at a crucial meeting of the electoral board in Oxford to find that it had already been decided to offer the chair to another candidate and of his ability to persuade his colleagues to change their decision. He goes on to describe Florey's frustration during his early years in the Sir William Dunn School of Pathology, his fight for what would now seem to be trivial sums of money for research and his disappointment at finding that the great Nuffield benefaction of 1936 would provide a rich endowment for clinical medicine in Oxford, but do nothing for the pre-clinical departments. But Florey rapidly invigorated what was a nearly moribund Department. He continued his work on mucus secretion and on lymph flow and the function of the lymphatic system. A later outcome of his interest in lymph, when labelling with radioisotopes and other new techniques had become available, was the important discovery by J.L. Gowans of the circulation of the lymphocyte.

Florey's work in these fields, often requiring surgery of great skill and designed to obtain unambiguous answers to straightforward questions, virtually established the subject of experimental pathology in this country. This was one of his great achievements.

The book describes how the research on mucus secretion, which originated from anxiety about his own dyspepsia, led indirectly to penicillin. While still at Cambridge, Florey had become interested in Fleming's lysozyme, probably because the presence of this bacteriolytic enzyme in intestinal mucus raised the question whether lysozyme, or a related antibacterial enzyme, was responsible for the protective action of the mucus. His wish to have lysozyme purified seems to have prompted the thought that experimental pathology would benefit from a collaboration of pathologists with chemists or biochemists. But it was only after he became Professor of Pathology in Oxford that he succeeded in obtaining the funds for a biochemist to work in his Department. It was thus that Ernst Chain, a Jewish refugee from Hitler's Germany who had just finished his PhD at Cambridge and was warmly recommended by Gowland Hopkins, came to the Sir William Dunn School of Pathology in 1935. He worked at first on the mode of action of snake venoms and then, at Florey's suggestion, he turned his attention, with L.A. Epstein, to the mode

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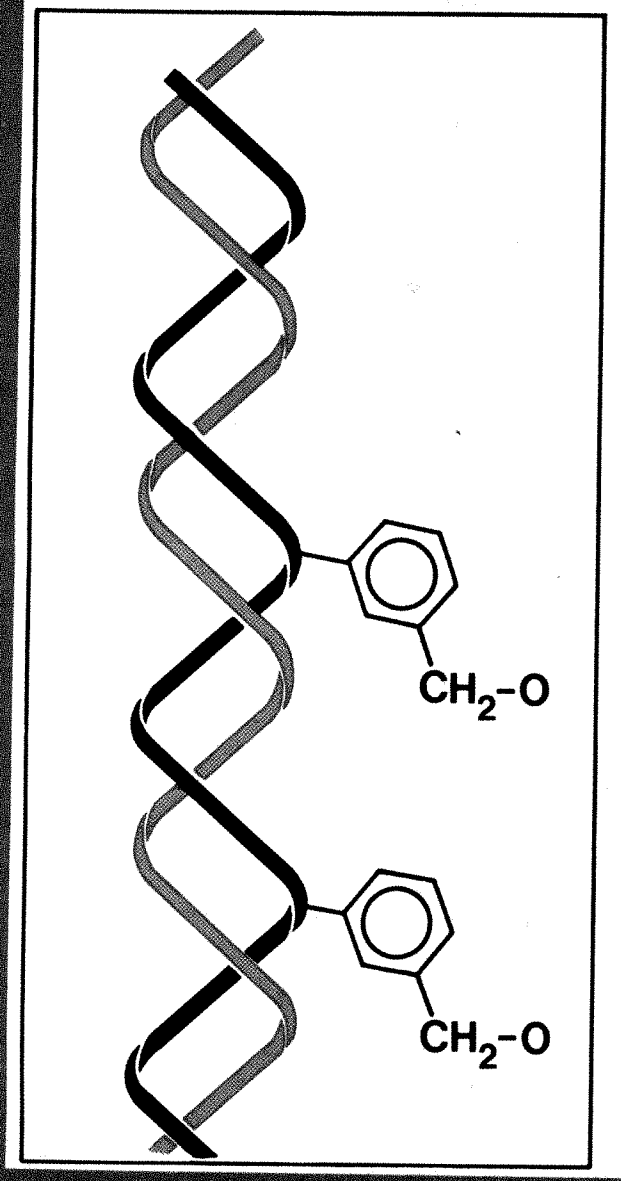
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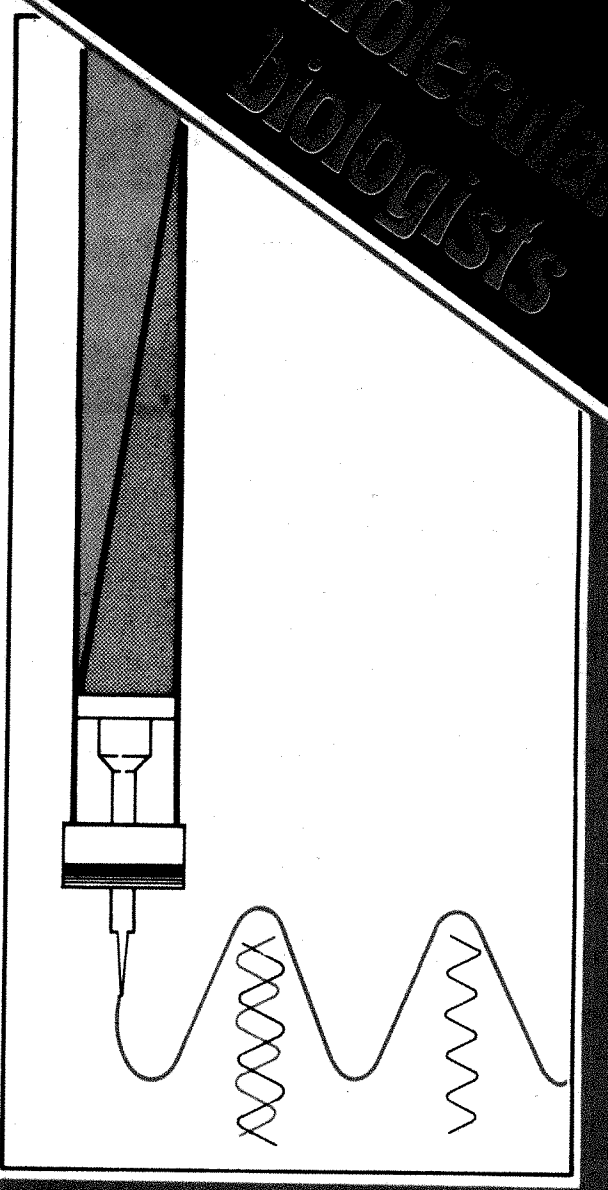
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Howard Florey

of action of lysozyme.

It has never been possible to write a history of penicillin that would have satisfied all of those who were closely concerned. But in most of its essential features Gwyn Macfarlane's well documented version probably comes as near to the truth as anyone is now likely to get, and it is only his treatment of some of the chemical studies which is open to question. He devotes one chapter to Alexander Fleming and the extraordinary conjunction of chance events (analysed by Ronald Hare in *The Birth of Penicillin;* George Allen and Unwin: London, 1970) that made it possible for him to discover penicillin in 1928. Why Fleming made no attempt to test its ability to cure bacterial infections in mice, as he could have done, is a question to which he never gave a satisfactory answer. There is no evidence for the suggestion of André Maurois, his biographer, that he was actively discouraged by his chief, Almroth Wright. His claim that he lacked chemical help is unconvincing, because he showed little interest in the work of two young assistants, Ridley and Craddock, who prepared crude extracts of penicillin, and none at all in the later and soon abandoned attempt at isolation by Raistrick, Clutterbuck and Lovell. It seems that the idea of using penicillin as a systemic chemotherapeutic agent simply did not occur to him, nor did it occur to such influential figures as Wright and Topley But Gwyn Macfarlane rightly says "Fleming should not be criticised for what he did not do" and concludes that his genius for discovery was not accompanied by the sort of character needed to exploit what he found.

Ten years after Fleming's discovery, an investigation of penicillin was begun in the Sir William Dunn School of Pathology, as Chain's work on the substrate of lysozyme led him to make a survey of the older literature on bacteriolytic substances. "Dr Chain and I", wrote Florey, "had many discussions on the possible interest of a systematic and thorough study of the known natural antibacterial substances"; and later he said: "eventually he proposed, and I agreed to go along with it, that we should make a thorough investigation of antibacterial substances. This is why we looked at penicillin". Chain's biochemical interest in penicillin was aroused by its reported instability and Florey was struck by its activity against the staphylococcus, against which the sulphonamides had proved relatively ineffective. However, penicillin was only one of three substances first chosen for study and was at first given no particular prominence. Gwyn Macfarlane leans towards the view that thoughts of "suffering humanity" lay behind the decision to work on penicillin. Florey, however, has stated categorically that this was not the mainspring of the motivation, which was scientific interest, and he did not fail to acknowledge the role of chance: "We happened to hit on an antibiotic which worked in man. We could have worked with hundreds of others and they would have been chemical curiosities".

Florey always maintained that the success of the penicillin project itself, which seemed at the time to be almost miraculous, was due to the combined work of a small group: "I am quite clear that nothing would have gone along except for a group of five or six people". Gwyn Macfarlane gives handsome bouquets to all those concerned in a detailed account of the extraction of penicillin and the dramatic results obtained from well-planned experiments by Florey, first in mice and then in man. But he rightly concludes that it was only Florey's drive and personality that held the group together, and enabled difficulties to be overcome which at first seemed insurmountable in war-time Britain.

Chain made no secret of his view that he had received too little credit and reward for his part in the penicillin story and that the Medical Research Council was guilty of a major error of judgement in not obtaining patent protection for the Oxford work on penicillin. But although it is now widely accepted that a policy which excludes the products of academic medical research from patent cover is a form of idealism that the country cannot afford, this was not so before and during the War. At Florey's suggestion, Chain went to Edward Mellanby, then Secretary of the Medical Research Council. However, Mellanby, who seems to have regarded him as an alien and troublesome personality, was unmoved. The official attitude became the subject of public criticism, but this may have been irrelevant, at least in relation to the achievements in Oxford, because it is by no means certain that a rewarding patent could then have been obtained. Although the results with penicillin were new and far-reaching, penicillin itself was not new and it had not been characterised chemically. The methods used in its production were soon to be superseded by others in America. The most important unpublished step in its

purification (extraction as a salt into water from an organic solvent), which was suggested in Oxford by N.G. Heatley, had already been taken (unknown to him) in 1934 by Lewis Holt, a chemist at St Mary's Hospital whose work was never mentioned by Fleming. Moreover, the penicillin produced later in the United States for clinical use (benzylpenicillin) differed in its side-chain from the penicillin then being studied in Oxford.

The book also contains comments on another aspect of this question. After the clinical trials at Oxford in 1941 Florey decided that American help would be essential for the war-time production of penicillin in quantity. With the agreement of the Medical Research Council and financial support of the Rockefeller Foundation, he and Heatley went to the United States to try to interest the American pharmaceutical industry. Penicillin, then only produced in minute quantities, was far from easy to sell, but Heatley was invited to the Fermentation Division of the Northern Regional Research Laboratory in Peoria to help get a project started. He worked with A.J. Moyer, described in the book as "a fervent anti-British isolationist" with a "positive flair for making moulds do what was wanted". Moyer and Heatley had agreed on a joint publication, but the paper which eventually appeared was in Moyer's name alone. During this time the outlook for penicillin production was revolutionised by the American decision to add corn-steep liquor to the growth medium and to use deep fermentation, and these advances became the subject of American patents. British firms were understandably irked when they discovered that they were required to pay royalties to America to produce a substance that was essentially a British discovery. But equity and patent law may sometimes diverge. At the root of the problem was the fact that the official pre-War world in Britain was entirely unprepared for a situation of the kind that penicillin threw up. And there is, of course, another side to the matter. Without American help it is inconceivable that enough penicillin would have been produced, as it was, to treat all serious battle casualties, British and American, when D-day came in Europe.

The penultimate chapter of the book is headed "Palmam qui meruit ferat", a phrase used by Almroth Wright in a letter to *The Times* on 31 August 1942 firmly placing a laurel wreath on Fleming's brow. The nature of the publicity that followed can be attributed partly to Florey's refusal to talk to the press, partly to the welcome given to the press by Fleming and others at St Mary's Hospital and partly to the unwillingness or inability of popular writers to acquaint themselves with the facts in the scientific literature. One of these stories, written by a bacteriologist at St Mary's and more ludicrous than others only in the extent of its fabrication, attributed virtually all that had been done in Oxford to Fleming himself. The source of this myth is uncertain, but Gwyn Macfarlane remarks that behind Fleming were Almroth Wright "intensely ambitious for his department" and Lord Moran, Dean of St Mary's Hospital Medical School "Whose dearest wish was for its honour, glory, and financial security" and suspects that an efficient Public Relations Officer was active somewhere in the organisation. Fleming himself is said to have derived an impish glee from the more bizarre stories as he collected cuttings, a reaction that might have been understandable had he not been gaining worldwide gratitude from their publication.

Florey complained of this situation to Edward Mellanby who replied that it was ephemeral and of little consequence and that "in time even the public will realise that in the development of this story of penicillin, the thing that has mattered most has been the persistent and highly meritorious work of your laboratory". There is now now reason to think that the publicity has ever affected the judgement of scientific bodies of any consequence, but its effect on the public has been far from ephemeral.

Gwyn Macfarlane finally summarises some of the qualities which combined to make Florey an outstanding figure: his great energy and enthusiasm for research; his skill as an experimenter and commitment to experiments that gave clearcut results; his judgement and sense of direction; and his complete intellectual integrity, never clouded by vanity. His colleagues who came to like and admire him will remember that behind a deep reserve there were kindness and consideration for their welfare. It is to be hoped that this well documented book will make his qualities and great contributions to science and medicine more widely known. □

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Olduvai record

J. Desmond Clark

Olduvai Gorge: My Search for Early Man. By Mary Leakey. Pp. 181. (Collins: London, 1979.) £6.50.

EAST AFRICA remains unsurpassed in the wealth of discoveries and the understanding these provide about the biological and cultural evolution of our own species. It is no exaggeration to say that the very impressive record of hominid fossils and cultural remains in their palaeo-environmental setting that has come from East Africa and the Horn over the past twenty years, has revolutionised our knowledge of human phylogeny. The hundreds of fossil hominid remains recovered, often remarkably complete and well preserved, and the circumstances of their preservation have made it possible to identify some of the behavioural adaptations that underlie the beginnings of tool-making and the ensuing intellectual development manifest in the increasing complexity of the tool-kits and the economic strategies that gave rise to them.

A very great deal of what is known today results from the lifetime devoted by Louis and Mary Leakey to their search for early man — a search which has completely vindicated Louis' claim, first made when he was an undergraduate at Cambridge in the 1920s, that a wealth of evidence awaited discovery in the deep sedimentary sequences of the Great Rift Valley. It was really the first full season's work in 1935 at the Olduvai Gorge in northern Tanzania, which was also Mary Leakey's introduction to fieldwork in the African Quaternary, that was the first demonstration of the correctness of Louis' conviction. It was also the first manifestation of his shrewdness — some would say his luck — in having concentrated on the Rift which, as can now be seen, is probably the most significant region in the world for preservation of the Plio-Pleistocene record that spans the emergence and early development of man the tool-maker.

The fossil, geological and cultural evidence is unique, both in its completeness and in the relatively minor disturbance that it has suffered. This has made it possible to reconstruct the changing climatic and ecological conditions under which hominid evolution took place, to find and excavate the activity places of the hominids, and to date all these events. In the 1930s, there was no way of knowing how old the then earliest fossils and stone tools were. At that time Keith's 'guesstimate' of 200,000 to 400,000 years did not seem unreasonable to many and, indeed, most people then thought in terms of half a million or so years for the beginning of the Pleistocene. Now, K/Ar, uranium fission-track dating and the palaeomagnetic reversal chronology show that the earliest stone tools are about 2 million years old; that the earliest, well-known hominid (*Australopithecus afarensis*) is between 3 and 4 million years old; and that even earlier, but fragmentary, remains are present in the East African sedimentary record. From the 1960s onwards, a number of interdisciplinary and international teams have been working in the East African and Ethiopian sections of the Rift and on the adjacent parts of the plateau — for example, at Laetoli, Olduvai, Omo, East Turkana, Hadar, and on the

Ethiopian Plateau. The discoveries and information that this work is producing are of paramount significance and there is every expectation that, as the research continues, an even greater wealth of material, detail and clarification will result.

Dr Mary Leakey's contribution to all this is both very substantial and outstanding in its importance. It was she who pioneered and introduced to Pleistocene prehistory the methods of excavation, recording and recovery that are common practice today. A meticulously careful and observant fieldworker, Mary Leakey found that activity areas were still preserved within the fine-grained sedimentary sequences in the East African Rift where she and Louis worked. She was also the first to demonstrate the existence of these early sites and to apply to them the methods used by archaeologists in the excavation of settlements of much more recent times. By exposing the whole, or large parts of an occupation area to give a complete visual perception of the relationships between all the imperishable residues — artefacts, bone waste, features, hominids, and so on — in their palaeotopographical setting, palaeoanthropologists now have the opportunity of making direct comparisons with the residues resulting from various accumulating and dispersing agencies — geological, chemical, animal or human — and therefore of developing explanatory models for early hominid behaviour.

Since 1959, when she found the first hominid cranium (*Australopithecus boisei*) in Olduvai Gorge, Mary Leakey has worked there almost continuously; and since 1968, she has made her permanent home at the Gorge. The scientific results of her work are recorded in the classic Volume 3 in the Olduvai Gorge Series (*Olduvai Gorge: Excavation in Beds I and II, 1960-1963;* Cambridge University Press, 1971) which is shortly to be followed by a volume on the discoveries in Beds III and IV and the later sediments. Obviously, such works are not the place in which to record some of the more personal events of everyday life at Olduvai — always exciting and packed with activity.

The present book is an attempt to make up for this by telling something of camp life at Olduvai and the way the work there has progressed through her own systematic fieldwork and that of the team of collaborators who have been working with her on the geology, palaeontology and chronology of the Olduvai sedimentary sequence that began two million years ago. For someone wishing for an overall appreciation of the setting in the Serengeti, a record of the main discoveries with something of their significance and an account of some of those involved with the author in the work at Olduvai, the book admittedly fills a need. It is divided into eight chapters, the first seven dealing with the early discovery of the Gorge; camp life; geology, dating and excavation methods; the stratigraphic sequence of sites in their geological setting; the search for the hominids; the nature of the stone artefact assemblages, with a very usefull illustrated glossary of terms; the associated animal remains; and the evolutionary changes which the Olduvai record shows that some of these underwent.



Mary Leakey at the site of WK East in Bed IV at Olduvai

Photo: J. Desmond Clark, 1972

The book, however, will not entirely satisfy the reader who looks for a more personal account of the life and work at Olduvai — the problems, stresses, excitements, frustrations and successes of the author's life and search. Her interest in the natural setting, the vegetation and the animals is readily apparent but some readers might, for example, have appreciated a description of the view from the camp dining room following the remark (page 23) that it is magnificent. Similarly, more might have been made of the opportunity to describe the Masai way of life. The layman would also, no doubt, have found it useful to have had more of the scientific names of the animals, and so on, explained in chapter 7.

On the other hand, the description of the prehistoric living sites and stone tools well conveys the author's particular interest in this part of the work and describes the way in which a living site is laboriously uncovered. Also, some of the excitement and rewards of the work are conveyed by the account of the stone circle which portrays the earliest man-made structure yet known and which was found at the base of the more than 350 foot sedimentary sequence, and also by the description of the more enigmatic "pits" on a later site. More space might have been given to an account of the butchery sites but prehistorians will find valuable the previously unpublished data on Beds III and IV and the later series of beds.

Regrettably, the hominid footprints found at Laetoli receive hardly more than passing mention and, even though Laetoli is a short way distant to the south of Olduvai, more space might surely have been devoted to this momentous discovery which provides proof of the bipedal nature of Pliocene man.

In the concluding chapter, Dr Leakey discusses the significance of the East African evidence and the great potential that exists there for increasing understanding of the ways of life of the earliest hominids. As she points out, the evidence coming from the African continent is today the most complete for this time period anywhere in the world; this can, in part, be explained by the unique circumstances of preservation and the continuity of research there. The opportunities for fieldwork as well as the circumstances under which fossils were preserved and the possibilities of dating them have not been so good in the Asian tropics. It is, nevertheless, essential to know if an equally long record of hominid evolution also exists there; and it is to be hoped that the field studies now under way will provide at least part of the answer, through the kind of teamwork which has proved so successful at the Olduvai Gorge and which is revealed in this book.

Although the book does not really do justice either to the enthusiasm or the known achievements of its author, it does contain much of interest to all kinds of readers. It remains a record of more than forty years of unparallelled devotion to the search for early man. □

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Memories of Sherrington

C.G. Phillips

Sherrington: His Life and Thought. By J. C. Eccles and W. C. Gibson. Pp. 269. (Springer International: Berlin, Heidelberg and New York, 1979.) DM34.

SHERRINGTON died in 1952. Within ten years a young collaborator of Ragnar Granit's was confessing that his generation knew nothing about him. Granit's answer was that many of the conceptual foundations of their research were created in Sherrington's mind. His *oevre* is remarkable for its sheer coherence. Discoveries and rediscoveries fall into place in an almost continuous narrative, as can be seen in Denny-Brown's *Selected Writings of Sir Charles Sherrington,* now reprinted by Oxford University Press for the Guarantors of the journal *Brain.* These, together with *The Integrative Action of the Nervous System* (1906) and the last chapter of *Reflex Activity of the Spinal Cord* (re-issued recently by Oxford University Press on D.P.C. Lloyd's initiative), are all readily available to anyone who wishes to know and value Sherrington's achievement. He was evidently concerned to leave his scientific testament in good order. Many will wonder at his highly individual literary style, seemingly so contorted in places and contrasting so strongly with the plain English in which he wrote the letters and reminiscences now published in Eccles' and Gibson's book. One has to remember that he was striving to express ideas which were not yet familiar to his readers. R.S. Creed in his memoir explains that Sherrington's first care was to escape ambiguity and to be concise, rather than to be immediately intelligible. The contortions were not casualties of confused thinking, but the result of deliberate rewriting, often delayed by the search for some crucial word. On these foundations neuroscientists are still building, usually as unconsciously as Granit's pupil. Though modest in manner, Sherrington was not quite without vanity: Denny-Brown has told how he "shyly" produced Hamburger's comment (which prefaces *Selected Writings*) that anyone who had witnessed his demonstration of reciprocal inhibition in a monkey at the Fourth International Congress of Physiologists in 1898 "a enrichi sa connaissance pour toujours". But in his preface to the re-issue of the *Integrative Action* (1947: extracts reprinted by Eccles and Gibson) he referred to it as "my rather elderly book". To J.F. Fulton he wrote (when he had just retired, in his late seventies): "Thank you for mentioning again that the old *Integrative Action* still floats. I know how generously you regard it, and am very grateful. But looked at rigidly I cannot but recognise that it has "had its day". Its best future is for what lives of it to be incorporated — unobtrusively — into what is written with similar scope, now and after, for a time". At about the same period, in his review in *Nature* of Romón y Cajal's autobiography, he quoted the "wistful" passage in which his old friend, whose structural work on the neurone had always complemented his own physiological work, accepted that "the facts associated at first with the name of a particular man end by being anonymous, lost for ever in the ocean of Universal Science. The monograph impregnated with individual human quality becomes incorporated,stripped of sentiments, in the abstract doctrine of the general treatise. To the hot sun of actuality will succeed the cold beams of the history of learning". Sherrington's comment: "We may express this in short by saying that, as Aristotle thought of the soul, a scientific writing has no personal immortality". Yet active scientists do sometimes go to the writings of their forebears for inspiration — a *creative* act of ancestor-worship.

C.E.R. Sherrington, in his Memories now published by Eccles and Gibson, wrote that his father was averse to the idea of any "extensive biography". Sherrington's pupils have respected this wish. In formal memoirs, written at the time of his death, they have recorded the facts of his career and the people who helped and influenced him. Of these memoirs, that by E.G.T. Liddell, in the Royal Society's series, is the acknowledged masterpiece. Liddell's homage took the form of a remarkable book, *Discovery of Reflexes,* in which, with the insights of a neuroscientist as well as those of a scholar who reads seventeenth-century Latin as well as French and German, he painted a splendid landscape of knowledge and ideas about the nervous system at the time when Sherrington began to publish in 1884. This sets in relief the veritably Harveian revolution which Sherrington brought about. Ragnar Granit's *Charles Scott Sherrington: An Appraisal* tells of the modern experimental exploitation of Sherrington's seminal discoveries and concepts: the synapse; central inhibition as an active process; convergence and interaction of excitatory and inhibitory synaptic inputs at the neuronal membrane; receptor organs in muscle and their reflex actions, as proof of their continuing vitality. Eccles and Gibson have also respected Sherrington's wish, though a sentence at the beginning of their Preface (not to mention "the definitive Sherrington biography" proclaimed by the publisher's dust-jacket blurb) might have alarmed him. "More and more, students of history are calling for creative writing on the whole man, particularly when he is a genius. Those interested in the genesis of ideas want to know the settings for discoveries and the relevant circumstances which ushered in new truths and insights". "Creative writing on the whole man" suggests imaginative reconstruction of feelings and motives and of intimate personal relationships. His visits to Eastbourne left Liddell in no doubt about Sherrington's feelings in his very old age. "Hardly ever, he felt, can a biographer discern and interpret the inner thoughts which have moved the mind of another man years before. The mind made the man, and its achievements alone are worth recording". But Eccles and Gibson's wholly delightful book is not that sort of biography. In the first place, getting on for one-third of the words are those of Sherrington himself, a charming anthology of writings relating largely to life outside the laboratory. In the second, the well-known landmarks of his career are freshly illuminated by quotation from a large collection of letters, which, together with other Sherringtoniana, are housed in Professor Gibson's Department of the History of Science and Medicine in the University of British Columbia. Thirdly, both authors worked in Sherrington's laboratory at Oxford, and now add their personal witness to that of the others who shared in the action during his last years, when, in his seventies, he had steered his reflex experiments away from their significance as items of adaptive behaviour and into the neuronal basis of their physiological production and execution, and had brought them to their zenith of technical refinement and quantitative precision.

The Preface says that the book will concentrate on themes that have been "but lightly touched on" by previous writers. One only of its promises is not performed: one finds nothing about any analysis by Sherrington of the lack of productivity of British medical science at the end of the nineteenth century. For readers whose first love is to do experiments, Eccles' story of his exciting years with Sherrington in the 1920s and 1930s will be the highlight of the book. They were counting the muscle-fibres in the motor units and estimating their average contractile force. (The cautionary tale of the "muscle angle" comes in here). The motor nerves had to be emptied of afferent axons by antecedent dorsal root ganglionectomy. Sherrington was not only skilled in this delicate operation, but was also responsible for the perfectly circular and uncrinkled appearance of the myelinated motor axons in transverse sections; he dehydrated the splinted nerves overnight in his bathroom at home, and spent many happy hours in teasing nerves in order to prove the branching of motor axons. They noted the two peaks in the axon diameter spectrum, but inexplicably, in view of their awareness of the motor innervation of muscle spindles and of Sherrington's discussion of its possible significance in his Linacre Lecture

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in 1924, failed to suggest that the smaller-calibre group was fusimotor. Then came the long series of experiments on the interaction of excitatory and inhibitory reflex imputs, testing Sherrington's already-published theories and leading up to their culminating statement in the final chapter of *Reflex Activity of the Spinal Cord*. The atmosphere of the laboratory comes to life as we learn how they recited Keats and Bridges during the routine preparations, how Sherrington spoke of such things as his belief that Verocchio's studio was like a modern research laboratory, and how, on those two strenuous days each week, they missed lunch but went into the town for an enormous afternoon tea before the final recording session.

Eccles' involvement in the mind-brain problem is well known, most recently from his book with K.R. Popper (*The Self and Its Brain;* Springer: Berlin; for review, see *Nature,* **272**, 770; 1978), and his Gifford Lectures have also been published by Springer this year as *The Human Mystery*. Sherrington thought deeply about the problem in his last years. In 1922, in his Presidential Address to the British Association (reprinted in an Appendix as *The Times'* report), he spoke in evolutionary terms of the "twilit emergence of mind from non-mind, which is repeated in the individual life-history". His Rede Lecture, *The Brain and its Mechanism* (1933) was "eagerly read" in the laboratory "as giving some glimpse of the philosophical thinking he had been developing unbeknown to us". In his Gifford Lectures, *Man on his Nature*, first published in 1940, and about which, also, he was "secretive" (or dismissive: to Fulton — "I fear they are *not* interesting") he developed these themes in a biological epic of Homeric stature, its whole conception and wealth of illustration the product, as Eccles well says, of one of the most cultivated minds of the modern world. The Lectures were on Natural (as distinct from Revealed) Religion, and the audience, mainly of clergymen, can have received little comfort. Supernatural explanations of biological phenomena — heredity, ontogeny, adaptation for survival — had been replaced by natural explanations or shown to be replaceable in principle. Animate nature is amoral and much of it can only be valued by moral beings as loathsome. Yet, though mindless, its products adapt to pressures which threaten survival in ways which seem purposeful. Then comes the gradual emergence of "recognisable mind", linked with the appearance of bigger and more complex brains, and conferring increasing domination of the environment and of other species. Eventually there appear aesthetic and moral qualities which give hope that altruism will supplant predatory behaviour. "It may likely mean a human future led by women more eminently than by men". Yet neurone and synapse seem everywhere the same; and, in spite of the essential unity of bodily and mental life, analysis finds *two* modes of activity, mind and energy — irreducibly. Confronted with this twentieth-century epic, different readers will respond differently. Eccles' response, as shown in the passages he has chosen and in his commentaries, follows the lines of his well-known books, but differs for example from Granit's (we may think) equally valid one. One can agree that Sherrington "embraces as equals all the values", but be puzzled by the statement (page 156) that he "even exalts altruism



Sherrington in 1950, aged 93

Photo: Allan G. Chappelow

above truth". One hopes that readers will be encouraged to go to the source. Schrödinger wrote: "I cannot convey the grandeur of Sherrington's immortal book by quoting sentences; one has to read it oneself".

Though deadlocked about how mind and energy interact in the brain, and unwilling to propose any hypothesis, Sherrington had written (in the Rede Lecture) "I am not a defeatist, for I would urge active pursuit of the enquiry", though "it seems to me, speaking in all humility, that from time to time the biologist has to make plain how far or near he is from the answer". If "man, the best among us", finds out "how the brain does its thinking", he "will certainly try to improve its ways of doing so . . . We need not be prophets to foresee that then will come the long-told speedy extinction of man. The planet will then be re-liberated, free for the next era of animal domination". Today it seems that the same danger could come from psychotropic substances discovered by chemists, whose control of thought and behaviour might bring extinction before neuroscientists have had time to discover "how the brain does its thinking".

Professor Gibson's quotations from his collection of letters illuminate the whole range of Sherrington's activities. As a professor, he never had a secretary, but wrote his many letters by hand and kept his files himself, with help from his wife. He could rely on an extraordinary memory. His letter of 1947, describing how his pioneering use of diphtheria antitoxin saved the life of his eight-year-old nephew in 1893 or 1894, is reproduced in facsimile. Although genial he could be firm when necessary — as when the Dean at Liverpool, an anatomist, tried to cut physiology by half and pathology by a third to gain more time for anatomy. He helped many people, notably Harvey Cushing, Wilder Penfield, Hugh Cairns and Howard Florey, in their careers. Private donors gave hime money for research. In thanking the British Medical Association for financial support, he drew public attention to the £4,000 per annum which in 1907 was all the Government gave to the Royal Society to support research in all fields in the whole of the British Empire. "The sum is about the same in amount as — sad to say — the average yearly takings of a public-house in a poor quarter of this city". He spent much trouble on public education and in service on committees — writing articles for the *Encyclopaedia Britannica*, six chapters for a manual of school hygiene, reports on health in schools, on the importance of longer hours of sleep in public schools, on tests for sight and colour blindness, and even on the infectivity of oysters. Partly to study industrial fatigue he worked during the long vacation of 1915 (he was 58) in a munitions factory. No-one knew who he was, and the foreman offered to write him a good testimonial. Asked by Alan Gregg of the Rockefeller Foundation about the function of modern Oxford, he replied that it could no longer rely on its centuries-old skills in teaching what is known, but "must learn how to teach the *best attitude* to what is *not yet* known. This also may take centuries to acquire but we cannot escape this new challenge, nor do we want to".

Gibson has a fine chapter on Sherrington the Poet, illustrated by several sonnets and extracts from longer pieces. His work was taken seriously by Hardy, Edmund Gosse and Walter de la Mare. An appendix reprints his undergraduate essay on Longfellow. There is a good deal of correspondence about his lifelong collection of incunabula — another interest about which he was ordinarily extremely reticent. His gifts to the British Museum marked him as its "best benefactor in modern times". Then there is his hilarious account of Christmas at Grindelwald in 1887. And so on. Having read it all you will want to browse frequently. □

C.G. Phillips is Dr Lee's Professor of Anatomy at Oxford and Editor of Brain.

Early days of behaviourism

O.L. Zangwill

J.B. Watson: The Founder of Behaviourism. By David Cohen. Pp. 297. (Routledge and Kegan Paul: London and Boston, 1979.) £8.95.

THIS is a biography of Watson; not a study of the origins of behaviourism. Nonetheless, Dr Cohen's well researched and highly readable book tells us a good deal about the early days of behaviourism, a school of psychology which Watson himself once spoke of with evident pride as "this purely American production". His account will do much to demolish the still popular myth that behaviourism amounted to little more than the export of conditioned reflexes from Russia to the United States and the substitution of muscle twitches for mind as the subject-matter of psychology. At all events, in so far as conditioned reflexes are concerned, Watson owed distinctly less to Pavlov than to his contemporary and rival, Vladimir Bechterev, whose correspondence with Watson may still be seen at the Bechterev Institute in Leningrad. Moreover, Pavlov himself made no secret of his detestation of behaviourism and ridiculed its pretensions to be a scientific discipline. Pavlov, of course, was a militant physiologist imbued with the materialist philosophy which he had brought from Germany, whereas Watson was a psychologist through and through, concerned only to establish a viable science of human behaviour. It is not surprising that relations between them were cool.

John Broadus Watson was born in 1878 in Greenville, South Carolina. His father was an impecunious farmer and his mother a pillar of the local Baptist community and a Fundamentalist who evidently ruled her family with a rod of iron. Following graduation from Furman University in 1899, Watson proceeded to the University of Chicago, where he came strongly under the influence of James Rowland Angell, a sometime student of William James' who favoured a biological, or as it was then termed 'functionalist', approach to psychology in contrast to the 'structuralist' approach of Wundt, with its emphasis on classification and introspective method, which Titchener and other early experimental psychologists had exported from Germany. Although Watson came later to disown 'functionalism' almost as radically as 'structuralism', he seems to have retained towards Angell himself a deep and abiding respect.

On Angell's advice, Watson began his graduate work in the field of comparative psychology. His PhD thesis, published in 1903 as a monograph under the title of *Animal Intelligence*, was largely concerned with the growth of learning capacity in the white rat, with special reference to the medullation of the nervous system. (The neurological aspects of this work owed much to the Chicago neuroanatomist, H.H. Donaldson, who evidently thought highly of Watson and to whose personal generosity he owed its publication.) In this study, it is noteworthy that Watson had no hesitation in using the concepts and nomenclature of introspective psychology, and it belongs much more closely within the 'functionalist' tradition than any of Watson's later publications.

Watson continued with his animal work at Chicago for several years and some of it has proved surprisingly durable. With Harvey Carr, he worked on spatial orientation in the rat, with special reference to kinaesthesis and the sensory control of maze learning. At this time, too, he first made the acquaintance of Karl Lashley, then still a graduate student, and collaborated with him in some early studies of bird behaviour. He also came to know Robert Yerkes, then a young physiologist at Yale, who became a firm colleague and friend. From Dr Cohen's account, it might be surmised that, had Watson remained in Chicago, it is by no means impossible that the scandal which was later to drive him out of academic life would have been averted.

In 1908, Watson became a full Professor of Psychology at Johns Hopkins University, and it was at Baltimore that behaviourism was born. No doubt the sympathetic biological climate of this University, and more especially its Medical School, contributed to this happy event, but by far the most important determinant was the advent of Lashley as a PhD student in zoology and psychology in 1911. Although Watson's background in animal psychology and, above all, his total disillusionment with the current schools of experimental psychology, no doubt touched off the behaviourist revolution, without Lashley it seems unlikely that behaviourism would have evolved as a credible competitor or achieved a measure of real scientific distinction. It was these two men working in close association who virtually created the behaviourist movement.

What, then, was the behaviourist position? This Dr Cohen outlines in two substantial chapters, adequate though philosophically less sophisticated than the account given by Dr B.D. Mackenzie in his recent book on *Behaviourism and the Limits of Scientific Method* (Routledge and Kegan Paul: London, 1977). It may be briefly summarised as follows. First, Watson had already proclaimed that psychology is (or should be) "a purely experimental branch of natural science whose theoretical goal is the prediction and control ofhuman behaviour". There seems little doubt that Lashley was fully in accord with this view. Secondly, both men insisted that introspection, for so long viewed as indispensable to the analysis of mind, has no place in behaviourist psychology; nor is the value of its data dependent "on the readiness with which they lend themselves to interpretation in terms of consciousness". Lashley later devoted two important papers to justifying this contention. Thirdly, behaviourism was in keeping with traditional Darwinism in so far as it recognised no sharp distinction between the behaviour of animals and that of man. At the same time, it must be borne in mind that, despite the preoccupation with animal behaviour which seemed almost to obsess the behaviourists, it was the understanding of human behaviour which Watson came increasingly to regard as the primary objective of psychological science.

In his seven years at Johns Hopkins, Watson was somewhat less active in personal research but successful in building up a Department of high standing in its field. He was particularly interested in developing detailed observational study of the development of behaviour in young children, particularly their emotional reactions, in connection with which he drew inspiration not only from Pavlov but, more surprisingly, from Freud, whose observations on infantile sexuality par-



Watson at Johns Hopkins, testing an infant's reflexes



Photos: Johns Hopkins University Archives

ticularly impressed him. Much of this work was carried out by his graduate students, one of whom was Rosalie Rayner, later his second wife, and some of their work was reported in *Psychology from the Standpoint of a Behaviourist* (1919), by far Watson's best book. It is odd that an important paper on conditioned emotional reactions in children, written in collaboration with Rayner, is not cited in Watson' bibliography as given in the present book.

In 1920, Watson featured in a divorce case which brought him considerable notoriety and entailed his enforced resignation from his Professorship. The whole sorry story and its attendant circumstances are fully described by Dr Cohen and it is of some interest to note that E.B. Titchener, a conservative Englishman who for many years represented the Wundtian tradition in America and who had no use whatsoever for behaviourism, was one of the very few professional colleagues who stood by Watson after his fall from grace. In spite of Titchener's support, however, Watson was never again offered a University post and resigned himself to a career in advertising, in which, however, he seems to have been strikingly successful. He also wrote a number of popular articles concerned with child upbringing and education, some of which won the approval of Bertrand Russell, who had himself experienced academic rejection at first hand and was no stranger to the devastating smugness of many American academic institutions some fifty years ago.

The only serious scientific work which continued to engage Watson after his resignation from Johns Hopkins was his observational work on young children. Although much of this was undertaken by a devoted co-worker, Mary Cover Jones, the ideas were undoubtedly Watson's and the work resulted in several interesting, if desultory, publications. Today, this work is of interest largely as the forerunner of what has come to be known as behaviour therapy, which has become a major industry in clinical psychology. Watson, however, did no more than adumbrate the theory and methods of behaviour therapy; its systematic development is the work of others more recent.

Had Watson remained active in psychology, one may hazard the guess that behaviourism would have run a very different, and perhaps more fertile, course. In particular, the obsession with learning theory which infected American psychology in the 1940s and 1950s, and in attenuated form is still with us today, might have proved altogether less dispiriting. On the other hand, the neurological element in Watson's thinking, later developed with such distinction by Karl Lashley, might well have taken earlier and firmer root in American psychology. The advent of behaviour therapy, too, might well have taken place sooner, and been less disfigured by empty 'scientism' and doctrinaire rejection of Freud and all his works. Finally, a tradition of research on human development might have grown up in America well before Piaget became the monolithic institution that he is today. As a "purely American production", Watson's behaviourism had a genuine feel for biology and a healthy freshness and enterprise in tackling the problems of experiment in the sphere of mind. Like many other American products, unfortunately, Watsonian behaviourism failed to survive the Depression. □

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Skinnerian behaviour

Stuart Sutherland

The Shaping of a Behaviorist. By B. F. Skinner. Pp. 373. (Alfred A. Knopf; New York, 1979.) $12.95.

To the general public B. F. Skinner is perhaps the best known experimental psychologist. He has attained fame by repeatedly asseverating the principle that all behaviour is governed by reinforcement, and by his sinister but well intentioned suggestion that we should engineer everyone's environment from birth to produce desirable behaviour. The second volume of his autobiography, *The Shaping of a Behaviorist,* describes his life between the ages of 26, when he entered graduate school at Harvard, and 45, when he became a Harvard professor. The title is misleading: the book in fact fails to reveal the schedule of reinforcement that deflected the young Skinner from his interest in English literature and turned him into a Behaviourist so extreme that he denied the existence of mental events. Unfortunately he abides by his own precepts, and gives no hint either of his own feelings and emotions or of those which actuated the numerous colleagues, acquaintances and girl friends who appear in these pages. In consequence, the book resembles a set of scientific protocols in which events are meticulously recorded but all speculation about cause and effect is avoided.

Skinner's professional success is in part due to his capacity for disciplining himself and for making the utmost use of his talents. He seems rarely to have had an idea that did not eventually appear in print: his book on verbal behaviour was planned ten years before it was written. His substitute for prayer was to write stern notes to himself: when he was thirty he prepared a document entitled "Plan of the Campaign for the Years 30—60" which ends with the frank acknowledgement "Plan for the Years 60—(?). These are beyond my present control". To explain how such elaborate exercises in planned self-control are learned would be a nice challenge to his own theory.

The period of his life described was by no means an easy one. It is true that he gained a prestigious Junior Fellowship at Harvard, but finding academic positions in the Depression was difficult and he was forced to exile himself to the University of Minnesota and subsequently to Indiana before returning triumphantly as a full professor to Harvard in 1948. During the War he attempted to train pigeons to guide a missile on to its target, but the idea was never taken up by the military authorities. He brought his second daughter up in a specially designed crib, but his attempts to market the "Heir Conditioner" were unsuccessful. Moreover, much of his early work on animal learning met with little acclaim and was overshadowed by the grandiose theorising of Hull and Tolman.

From an early age Skinner set himself against theorising: he was interested only in finding some behaviour that would vary in a quantitative way with changes in the controlling factors. For him the discovery of such a lawful relationship was enough, and he showed little concern over the novelty or importance of his findings: indeed in the absence of a theory, there can be no criteria for what is an important finding.

Nor did Skinner open up any



B. F. Skinner

Photo: Christopher S. Johnson

fundamentally new areas of investigation, with the exception of the effects of different schedules of reinforcement: even this work led less to the discovery of new principles than to the elaboration of his basic postulate of reinforcement. If a rat is not rewarded for pressing a bar until one minute has elapsed since the last reward, it learns to press infrequently for a time after reward is delivered and more frequently towards the end of the one minute interval. Skinner explained this result by pointing out that only bar presses made after one minute has elapsed will be reinforced, and hence the animal will come to give more responses after that interval and fewer immediately after reward; the nature of the timing processes that underlie such behaviour was of no interest to him.

Within the limits that Skinner imposed on his own thinking, he often displayed considerable ingenuity. He was amongst the first to distinguish clearly between the classically conditioned Pavlovian reflex and 'operant' responses in which reward is made contingent on the occurrence of the response. He also showed great skill in the design of apparatus for the automatic recording of behaviour, though at the expense of restricting the behaviour recorded. He does not make it altogether clear whether the famous Skinner box was an attempt to make the recording of behaviour more objective or whether it was a device to enable him to carry out more experiments with less effort.

It is interesting to speculate how Skinner's impoverished view of the task of psychology came to attract so wide a following. His insistence on quantitative results may have appealed to those who wanted psychology to attain the respectability of a science; his doctrine of the malleability of man fitted the prevailing ethos of self-improvement in America; his insistence that what was inside the organism was the province of physiology, whereas psychology should concentrate on the whole organism gave psychology status as an independent discipline; and his concentration on the role of reinforcement to the exclusion of all other problems obviated the difficulty of thinking about such complicated questions as how the organism stores or retrieves information.

Psychology is now a very different subject and the study of mental processes is once more fashionable. The next volume of Professor Skinner's autobiography will doubtless record how his fame amongst the general populace spread at the time his influence on the academic community was waning. One can only hope that he will relax his professional austerity sufficiently to tell us not only what happened but how he felt about it. □

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The Cardinal Sin

Ann M. Clarke
A. D. B. Clarke

Cyril Burt: Psychologist. By L. S. Hearnshaw. Pp. 370. (Hodder and Stoughton: London; Cornell University Press: Ithaca, New York, 1979.) £8.95.

THIS is a book about an eminent psychologist who committed the scientist's cardinal sin, deception and fraud, during a long productive life which brought him nearly all possible distinctions except Fellowship of the Royal Society. He is to be condemned not for the views he espoused, many of which we share albeit in a less extreme form, but for his irregular conduct over many years. On the penultimate page of Sir Cyril Burt's biography the author writes:

"It would be totally unfair for a final judgement on Burt to focus on his deceptions to the exclusion of all his positive achievements. He was not, perhaps, either by training or temperament a scientist. He was too impatient to reach, and too confident of having reached, firm conclusions which became for him very early in his career, articles of almost religious faith to be defended at all costs. Yet he brought to psychology many conspicuous gifts..."

Be that as it may, his major claim to fame certainly outside his profession, was his work on the heritability of intelligence, and in this connection many distinguished scientists on both sides of the Atlantic accepted his findings on the assumption that the data as reported were sound.

This beautifully written and well researched book records the life and works of an often charming, sometimes generous and kind, always erudite yet unscrupulous man who deserves a high place in science's rogues' gallery. In 1905, while reading classics at Oxford, he recorded in his diary "My purpose in life concerns primarily myself. It is to produce one perfect being for the universe", and went on to list the necessary virtues. Regrettably he failed to follow his own precepts. Shortly after graduation he was appointed assistant to Charles Sherrington at Liverpool. He remained there five years, commencing his research career and gathering his first data to prove that what he called "general cognitive ability" was innate. As Professor Hearnshaw notes:

"inadequate reporting and incautious conclusions mark this first incursion of Burt into the genetic field. We have here right at the beginning of his career, the seeds of later troubles."

In 1912, with a reputation already firmly established and on the recommendation of Spearman and Sherrington, Burt was appointed psychologist to the London County Council on a half-time basis. Spearman had written:

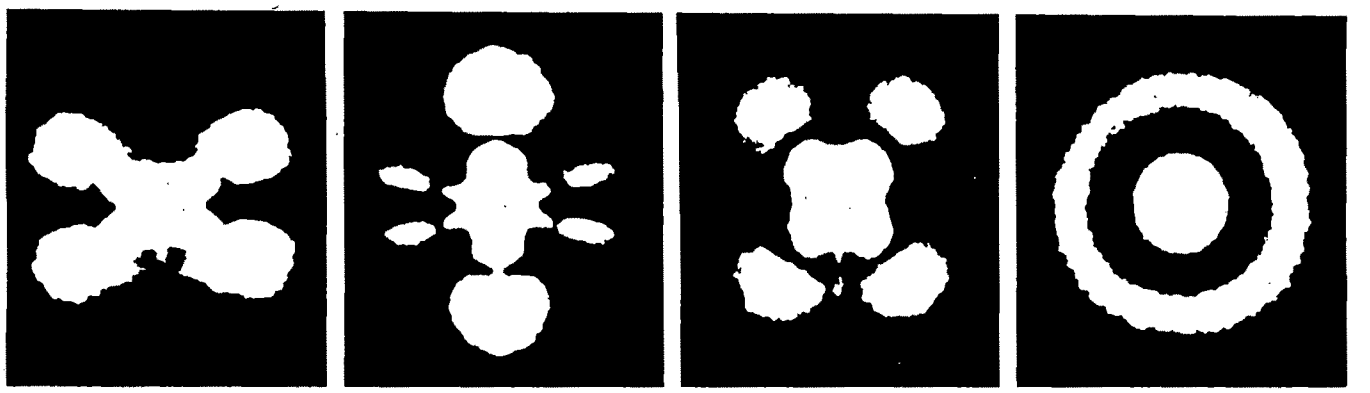
"I can add that he is considered by most experts to be the most brilliant and promising of the younger generation of psychologists in the British Isles...".

Burt seems to have worked indefatigably to collect data on abilities and attainments of London school children and on the development of mental and scholastic tests for their assessment.

Appointed in 1922 to a half-time post in the National Institute of Industrial Psychology to organise its vocational department, Burt remained there for two years and among other things taught some of his colleagues to 'estimate' the IQs of adults without actually testing them! In 1924 he was elected to a part-time Professorship of Educational Psychology in the then London Day Training College, later to become the Institute of Education.

In 1931, aged 48, Burt succeeded Charles Spearman in the Chair of Psychology at University College, London. During the next thirty years, factor analysis became his major interest. He began to make claims to priority as first user of this method, but these were at once retracted when Spearman challenged him. With the latter's death in 1945, however, Burt began to rewrite the history of these statistical techniques, dethroning Spearman as their originator. In a number of publications, especially from 1947 onwards, he began "to make claims about his own early work which were demonstrably contrary to the truth" (page 173). Far from having followed Spearman's lead, he now alleged that he had pursued a different route, and that he was in disagreement with Spearman from the start, a view belied by extant correspondence between them. Later "the claims got more and more extravagant". As Hearnshaw notes, there is a pathology here, of which we could ourselves offer further examples.

Burt retired from his Chair at University College in 1950, after unsuccessfully trying to influence the appointment of his successor. His subsequent attempts at interfering with the running of the Department ultimately led to his exclusion from the College on the decision of the Provost who had earlier warned the new incumbent against allowing Burt any access. The twenty-one years of retirement witnessed a surge of publications on a major theme, the importance of genetic factors in human ability and the unimportance of environment. His most influential papers were all published in journals with which he had a close personal connection. As founder and editor of *The British Journal of Statistical Psychology* from 1947 to 1963 and later as assistant editor, he used it as a frequent outlet. His gross abuse of editorial powers was to some extent known at the time, at least to people like



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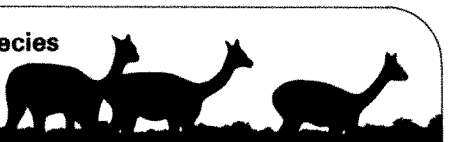
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Norman Myers



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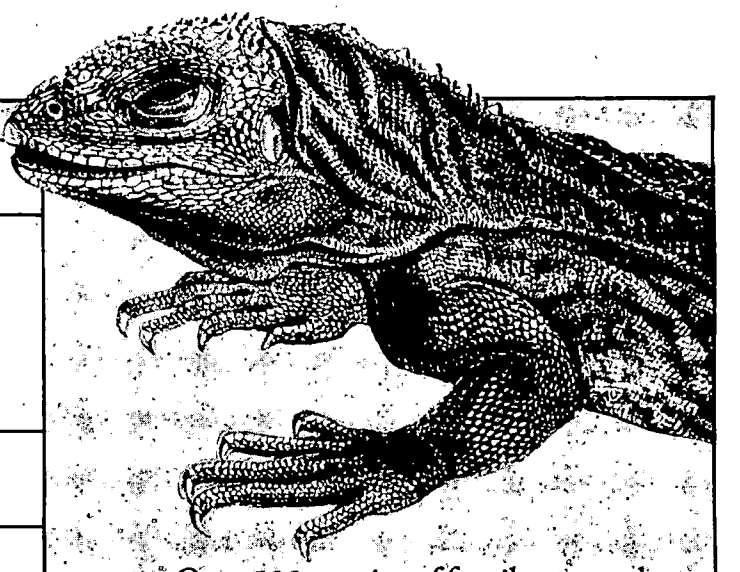
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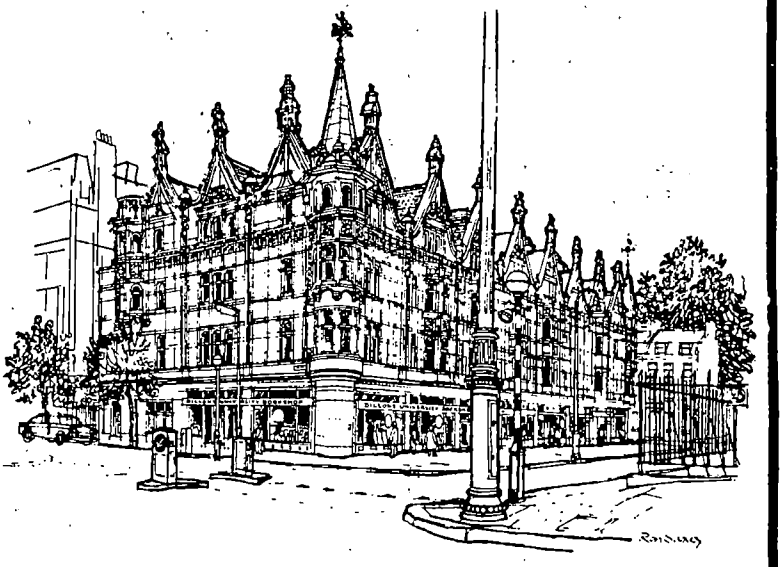
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ourselves around London. Apart from publishing 63 articles under his own name, he frequently altered authors' texts without permission, often misrepresenting their intention and adding favourable references to his own work. In addition "Of the more than forty 'persons' who contributed reviews, notes and letters... well over half are unidentifiable, and judging from the style and content were pseudonyms for Burt" (page 245).

Burt could also be kind and generous, as he usually was to us; Hearnshaw records many tributes from former colleagues and students. He could equally be autocratic and vicious towards, for example, a close colleague to whom he wrote a barrage of memoranda and letters pouring scorn on his research. All this to someone to whom staff and students were deeply attached, and upon whose devoted labours the functioning of the Department largely depended (page 143).

With his undoubtedly brilliant mind and wide educational and clinical experience, Burt became from the 1920s and onwards much in demand for evidence and advice on a variety of practical problems. Hearnshaw suggests that he influenced the developing pattern of English education, as well as contributing to policy in such fields as mental deficiency, delinquency and War Office selection procedures.

So far as any scientific theory is concerned it matters little whether Burt's claim to possess a vast store of data on the intellectual status of relatives was true; their poor quality and many anomalies in reporting were outlined in three independent publications in 1974. However, when in 1976 Dr Gillie of *The Sunday Times*, with our support, charged Burt with fraud there were strong protests. Such charges, particularly in connection with his sample of monozygotic twins reared apart (unique both in size and in extent of environmental differences, mainly reported in the 1950s and 60s) could, however, be made with impunity once Dr Gillie's fairly extensive enquiries had established that Burt's alleged collaborators, Margaret Howard and J. Conway, existed in no relevant records, nor were they known to colleagues at University College nor to his secretary/housekeeper. Space precludes a detailed account of Hearnshaw's later careful researches in connection with the charges; in his endeavour to be both brief and balanced, he has omitted many of Burt's conflicting stories to interested scholars during the last few years of his life, confining himself to a small but adequate sample.

Hearnshaw presents overwhelming evidence

"that neither Burt nor any of his alleged assistants carried out any field work after 1955, and it is probable that all his data, such as it was, had been collected prior to his retirement in 1950, most of it, indeed, prior to the Second World War. Burt certainly added no data himself after leaving University College, and there is no evidence of any contact or communication with any assistants" (page 239).

Moreover, he did not have, as he frequently alleged, material stored there.

The biographer acknowledges the possibility that the data simply did not exist, but writes:

"Assuming that the data were collected in the first instance, and there seems reasonable grounds for thinking that at least some was, what happened to it? The answer almost certainly, is that the bulk of it was destroyed during the war."



Cyril Burt

The evidence in support of this conclusion is less than convincing, and includes a letter from Burt to University College written from Aberystwyth in February 1941, requesting permission to store

"files of case-histories of defective, neurotic and delinquent children, rather large collections of children's drawings, compositions etc. systematically gathered while I still had access to London schools..." (page 248).

There is no mention of twin data nor any other material relating to intelligence test results for relatives. Whatever the truth of the matter, one thing is certain: as we pointed out over two years ago, the unknown Miss Conway did not double the size of the separated monozygotic twin sample, between 1955 and 1958, as 'she' and Burt claimed, nor were any further cases examined thereafter. Furthermore, it seems safe to assume that whatever data Burt may once have collected on various kinship correlations these, as M. McAskie (Hull) and more recently D. Dorfman (Iowa) have contended, were not available to him at the time of writing his seminal papers.

It is also clearly established that information on declining educational standards said to have been compiled by another unknown lady, Miss M. G. O'Connor, which included surveys carried out in 1955 and 1965 (discussed in the 1969 *Black Paper* and in the *Irish Journal of Education*) "were at least in part fabricated" (page 259).

Hearnshaw takes issue with those who like ourselves would dismiss Burt altogether on this evidence, pointing to the esteem with which he was held almost universally in the early stages of his career, and by many up to the time of his death. Among these were two of our former teachers, who we believe would never have expressed their approval had they known what is revealed in this book. The author alludes to Burt's use of short cuts, yet maintains that his contribution to applied psychology was important and must be taken into account in the final evaluation. We have reservations about this conclusion. For example, we have a little known report, published in 1924, in which he states that by means chiefly of psychological and educational tests, he had made an intensive study of backward children. "During the four weeks covered by my visit to Birmingham I personally tested 562 children. This was at an average rate of rather over ten minutes to every child". The psychological examination which he personally undertook used "chiefly the Binet-Simon tests of intelligence revised for English children". From our own extensive experience of testing such children we find this a disturbing example of taking short cuts, particularly as Burt was subsequently regarded as an authority on *The Subnormal Mind*. Later advances in this applied field in fact owed virtually nothing to him.

We cannot recommend too strongly this scholarly yet easily read biography. For this reason we refrain from comment on the well researched and fascinating suggestions concerning Burt's pathological personality and why he behaved as he did. This must have been a difficult book to write, partly because Burt was in Professor Cohen's words "a polymath of Renaissance dimensions"; partly because it was originally commissioned by Burt's sister who, after the public charges made against her brother, remained "convinced of Cyril's integrity"; and partly because of the heavy responsibility for sifting the evidence and arriving at an acceptable account of the life and works of an eminent psychologist who inspired devotion in some and distrust in others.

Two disturbing questions remain. First, why were Burt's deceptions unrecognised for so long? Second, is this case unique in our time? □

Ann M. Clarke is Reader in Educational Psychology in the Department of Educational Studies, and A. D. B. Clarke is Professor of Psychology at the University of Hull, UK.

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Phenomenon of human culture

Edmund Leach

Cultural Materialism: The Struggle for a Science of Culture. By Marvin Harris. Pp.381. (Random House: New York, 1979.) $15.

THIS is the sixth substantial book that Harris has published since the beginning of 1964. It would be unfair to say that to have read one is to have read them all but certainly there is a great deal of repetition. In the present case the author himself points out (page 77, footnote) that chapter 4, "The Scope of Cultural Materialist Theories", is a partial synopsis of his textbook *Culture, People, Nature: An Introduction to General Anthropology* (1971). Other familiar arguments derive from *The Rise of Anthropological Theory* (1968), *Cows, Pigs, Wars and Witches: The Riddles of Culture* (1974) and *Cannibals and Kings: The Origins of Culture* (1977), but have also appeared in various shorter productions. Second and third time round Harris has not modified any of his positions in response to the chorus of criticism from professional colleagues. Instead he stridently proclaims that he has been misrepresented and then counter-attacks in like measure.

The book is in two parts. Part I, "Cultural Materialism as a Research Strategy", summarises Harris' position and only just falls short of claiming that Harris himself will prove to be as significant for the history of anthropology as Darwin has been for the history of zoology. Part II, "The Alternatives", contains chapters labelled "Sociobiology and Biological Reductionism", "Dialectical Materialism", "Structuralism", "Structural Marxism", "Psychological and Cognitive Idealism", "Eclecticism", and "Obscurantism". Collectively these " 'isms" are all embracing and there are few contemporary anglophone or francophone social or cultural anthropologists of any reputation, either distinguished or undistinguished, who do not get an adverse mention somewhere. Indeed, Harris manages to convey the impression that all current forms of anthropological theorising other than his own are completely worthless. For anyone who already knows the facts of the matter most of this shadow-boxing is extremely boring but Marshall Sahlins, whose long review of *Cannibals and Kings* in the *New York Review of Books* provokes repeated anathemas, must be quite delighted to have got such a rise out of his victim.

It is useless for a reviewer to comment in any detail upon these exercises in academic abuse. Harris conducts a one-sided shouting match against named but inaudible opponents, but if one takes the trouble to discover what these opponents actually said it nearly always turns out either that the other side has much the stronger case or else that Harris' sarcasm completely misses the point. No doubt there are many of Harris' undergraduate readers who will only be too delighted to learn from a senior Professor that they need not bother to try to understand the mathematical, transformational intricasies of Lévi-Straussian structuralism because it is all a lot of nonsense anyway; but, to the initiated, all that Harris manages to demonstrate in this particular chapter is that he himself has completely misunderstood Lévi-Strauss.

The attack on the structural marxists has a more personal twist. In the first place Harris wants to persuade his readers that Cultural Materialism is a sort of improved Marxism with the Hegelian dialectic left out! But besides that, in attacking the 'idealism' of the structural marxists, he is also trying to get his own back on former colleague and pupil Marshall Sahlins and Jonathan Friedman, both of whom have treated Harris' earlier publications with mocking disrespect. But here again most readers are likely to feel that Harris gets much the worst of the argument. And so it goes on. No doubt some of the targets for Harris' boomerangs deserve to be hit, but his scorn is so indiscriminate that the only impression that survives is astonishment at Harris' personal vanity and lack of scholarly judgement.

So what is at issue? In part it is a very old argument indeed. Harris' favourite jargon which opposes 'emic' and 'etic' approaches to culture is only a rehash of the mediaeval disputations over the existence or non-existence of universals which divided the realists from the nominalists. Here Harris' support for an 'etic' position entails a realist universalist doctrine, which is curious in view of his otherwise passionate antipathy to the metaphysics of idealism in all its forms! In other respects Harris' creed is the unsophisticated materialism of the mid-nineteenth century, the standard definition of which is "the view that all facts (including facts about men's minds and wills and the course of human history) are causally dependent upon physical processes, or even reducable to them". Harris is obsessed with the idea that the anthropologist's essential task is to elucidate the causes which have produced the phenomena of human culture as they can now be observed and as they have occurred in history. He believes further that these causal sequences have been simple rather than complex and that the underlying causal principles can be discovered by *a priori* reasoning concerning energy inputs

and outputs without any reference to the fine details of historical or archaeological evidence. In Harris' view virtually all the diverse peculiarities of human culture can be "explained" by taking close note of such "empirical" factors as ecology, protein resources, and population density; but, above all, a universally valid economic law of nature will ensure that historical development will proceed in such a way that, in the long run, a minimax principle will govern the balance of cultural costs against cultural benefits. Harris seems to regard this principle as a sort of anthropologist's equivalent to the second law of thermodynamics; but, as Sahlins has pointed out, it is simply a value judgement among other value judgements and closely akin to the profit motive which so many of Harris' neighbours on Manhattan Island also consider to be a law of nature. Just how far Darwin was right in this regard on the issue of biological evolution is still an open question, but Harris' support of Adam Smith's earlier contention that, in free-for-all economic competition, efficiency will always win out against both politics and morality is mere mysticism.

Harris' professional colleagues disagree with him on a great variety of issues. I can mention only a few. Most of us would hold that in the absence of detailed historical records it is just as difficult to reconstruct the past as to predict the future, so that anthropologists will be well advised to devote their energies elsewhere. Harris' exercises in conjectural history are no doubt very satisfactory for that wide class of general reader whose members want to believe that everything is very orderly and simple and that everything that has ever happened in history is quite easily understood if only we apply the most simplistic principles of common sense; but it does not seem likely that there can be many members of the scholarly professions who would take that line.

Another major objection that has been raised against Harris' work is his irresponsible misuse of the limited historical evidence at his disposal. Any ordinary non-anthropological reader of Harris' books must get the impression that it is an unambiguous, fully established, historical fact that at the time of the Spanish conquest of Mexico the ordinary diet of ordinary Aztecs was desperately deficient in animal protein. Harris in fact claims that it is this dietetic deficiency which provides a "scientific" explanation for the uniquely elaborated institution of cannibalistic human sacrifice among the Aztec. But in fact there is no clear evidence that human sacrifice and cannibalism were closely associated among the Aztec or that the Aztec were ordinarily any more deficient in animal protein than most human populations at most periods of human history.

Complaints of this sort can be raised against almost the whole of Part I of Harris' book. He exhibits *Cultural Materialism* as a crudely simplified theory of law-bound social evolution which most professional anthropologists are likely to find even less convincing than that offered by Morgan in *Ancient Society* (1878). The theory is not derived from any ethnographic evidence; ethnography is only brought in as illustration, but often in such a slap-dash way as to be totally misleading. For example, around page 104, Harris is discussing his theory of the origin of the state, which is heavily dependent upon the theory put forward by Wittfogel in 1957 concerning the significance of large scale irrigation works for the formation of centralised forms of administration. "It seems likely", writes Harris, "that there was a causal relationship between the size and importance of irrigation systems and the degree of centralisation of political power". He then illustrates this thesis by the examples which Wittfogel himself used: Nile Valley, Yellow River, and so on. But how likely is likely? How large is large? Harris never tells his readers that the earliest large scale irrigation system so far identified by the archaeologists is in Highland New Guinea, a discovery which runs entirely counter to the whole of Harris' *a priori* argument!

Nor is it only on technical issues of anthropology and archaeology that Harris seems at times to be almost wilfully careless. Harris would like his readers to believe that he is well grounded in the philosophy of science. At pages 13-14 we are given a one paragraph summary of logical positivism which is described simply as "a movement which arose in Vienna" (no names; no dates). Two pages later we are told that the verification principle of the logical positivists has been "severely attacked in recent years" under the influence of "the English philosopher Karl Popper". Needless to say *Logik der Forschung* (1935) is cited only in the English edition of 1959. For a scholar who makes such a fuss about the importance of origins and causal sequences this is surely a very astonishing way of presenting the complicated history of the Vienna Circle of which Popper himself was, almost from the start, a very influential, though peripheral, member.

This book will not add to Harris' professional reputation but, regrettably, the cheap sarcasm which Harris directs against the serious arguments of his colleagues often makes a good read. Consequently it may well reinforce the illusion created by Harris' other books that social/cultural anthropology is not a serious academic pursuit at all and that explanation in these fields exists only at the level of Kipling's *Just So Stories*. Some anthropological writing is like that, but not all. □

Sir Edmund Leach is Professor Emeritus of Social Anthropology in the University of Cambridge, UK.

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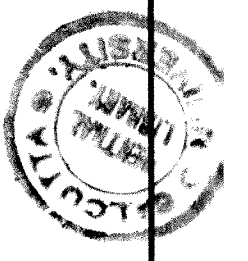
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Gaia and creative evolution

René Dubos

Gaia: A New Look at Life on Earth. By J.E. Lovelock. (Oxford University Press: Oxford and New York, 1979.) £4.95.

I HAVE always disliked the expression "Spaceship Earth" to designate our planet and I believe that it has led to faulty thinking about environmental problems. The word spaceship calls to mind a mechanical structure carrying a limited supply of fuel for a defined trip and with no possibility of significant change in design. The Earth, in contrast, has many attributes of a living evolving organism. It constantly converts solar energy into innumerable organic products and increases in biological complexity as it travels through space. I was therefore much interested when Jim Lovelock suggested in *New Scientist* in 1975 that the surface of the Earth behaves as a highly integrated organism capable of controlling its own composition and its environment. He used the name of the Greek Earth goddess Gaia to symbolise this complex biological behaviour of the Earth. Lovelock has now developed the Gaia hypothesis in a book that I have read with immense pleasure. I shall first briefly summarise its factual contents, then formulate a few questions in the hope that they will lead to a further development of the hypothesis.

It is not the first time that the Earth has been regarded as a living organism. In a book entitled *The Land Problem,* Otis T. Mason, one of the early American environmentalists, wrote in 1892, "Whatever our theory of its origin, the earth may be discussed as a living, thinking being . . .". The Gaia hypothesis is more concrete and can best be formulated in Lovelock's own words: "The physical and chemical condition of the surface of the Earth, of the atmosphere and of the oceans has been and is actively made fit and comfortable by the presence of life itself. This is in contrast to the conventional wisdom which held that life adapted to the planetary conditions as it and they evolved their separate ways.".

The Gaia concept has its origin in the fact that the chemical composition of our atmosphere is profoundly different from what it would be if it were determined only by lifeless physicochemical forces. In an Earth without life, purely physicochemical phenomena would produce for example an atmosphere containing approximately 98% carbon dioxide with very little if any nitrogen, whereas the corresponding figures for our atmosphere are approximately 0.03%, 79% and 21%. Lovelock presents numerous other examples of such profound departures from chemical equilibrium and postulates the existence of a global force which is able to bring about and keep fairly constant a highly improbable distribution of molecules. He believes that this hypothetical global force consists of the countless forms of life which create and maintain states of disequilibrium through cybernetic systems. Some of these are described in the book. As I do not have the competence to evaluate the validity of Lovelock's physicochemical arguments, I shall restate the problems he discusses in biological terms with which I am more familiar.

The fitness of an organism to its environment is, of course, an essential condition of its biological success and even survival. All living things seem to be endowed with a multiplicity of mechanisms that enable them to achieve fitness by undergoing adaptive changes in response to those of the environment — genetic changes that occur during the evolution of the species, physiological and anatomical changes that occur during the existence of each particular organism. In higher species and especially in humankind, these biological mechanisms are supplemented by adaptive social forces. Early in the present century, however, the Harvard physiologist L.J. Henderson pointed out that there was more to fitness than the adaptive potentialities of living things. As he claimed in his famous essay "The Fitness of the Environment," the achievement of fitness depends not only on adaptive mechanisms but also on the fortunate fact that the terrestrial environment has certain physicochemical attributes which happen to be just right for life. But this theory now seems essentially meaningless for the following reasons.

Many present forms of life would certainly be annihilated if the surface of the Earth were very different from what it is now. They could not adapt rapidly enough if the salinity, the acidity, the relative proportions of gases, minerals and organic substances — or any of the other physicochemical characteristics of the Earth — were to deviate far from their present values for any length of time. In other words, the present environment does indeed exhibit fitness for the present forms of life but — and this is what Henderson had overlooked — it would have been unsuited to those of the distant past. An atmosphere with 21% oxygen, for example, would have been extremely toxic for the earliest forms of life.

During the past 3.5×10^9 years, the global environment has progressively changed, probably as a consequence of the activities of living things, and living things also have undergone corresponding changes through a feedback process. The Gaia system postulated by Lovelock therefore seems be a result of co-evolution. Instead of listing the chemical examples of this creative process discussed by Lovelock, I shall quote a picturesque phrase taken from one of his recent articles: "The air we breathe can be thought of as like the fur of a cat and the shell of a snail, not living but made by living cells so as to protect them against an unfavorable environment.".

A few years ago, theoretical discussions were instigated by NASA to consider the possibility of making the planet Mars suitable for human life — which meant providing water, oxygen, moderate temperatures, protection from ultraviolet radiation, and so on. The general conclusion was that Mars could be made habitable only through the progressive introduction of living species capable of creating, over an immense period of time, more and more complex ecosystems similar to the ones which have evolved on Earth during more than 3×10^9 years. This analysis has helped us recognise the profound and innumerable changes that life had to bring about on the surface of primitive Earth to create, for present living things, the fitness of the terrestrial environment that L.J. Henderson had taken as the normal state of affairs.

According to the Gaia hypothesis, the Earth's biosphere, atmosphere, oceans and soil constitute a feedback or cybernetic system which seeks an optimal physical and chemical environment for life on this planet. At any given time, this system results in a relative constancy both in the composition of the environment and in the characteristics of living things. Repeatedly throughout his book, Lovelock refers to this equilibrium as "homeostasis" — a word invented by the Harvard physiologist, Walter B. Cannon, to denote the remarkable state of constancy in which living things can maintain themselves despite changes in their environment.

The word homeostasis, however, does not do full justice to the Gaia concept, which implies in addition that living things have profoundly transformed the surface of the Earth while themselves undergoing continuous changes, in a co-evolutionary process. Practically all the examples that Lovelock discusses refer, in fact, to creative evolution rather than to homeostatic reactions. For example, the accumulation of oxygen in the air which became significant 2×10^9 years ago (as a result of biological photosynthetic activities), probably destroyed many forms of life for which this gas was poisonous. In Lovelock's words, however, "Ingenuity triumphed and the danger was overcome, not in the human way by restoring the old order, but in the flexible Gaian way by adapting to change and converting a murderous intruder into a powerful friend.". Cybernetic mechanisms progressively brought about the emergence of biological species capable of living in the presence of oxygen and of using it for the production of energy. In this case, as in

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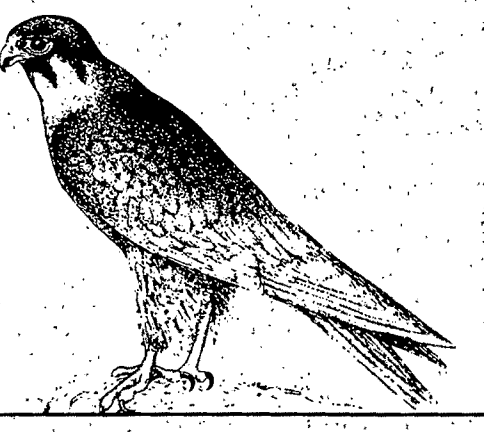
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most other environmental changes, the Gaian way was not an automatic homeostatic reaction but a creative co-evolutionary response.

It seems worth considering that the Gaian control results in global homeostasis only over a period of time which is short on the evolutionary scale. One figure will suffice to illustrate the magnitude of the terrestrial changes that are continuously caused by life. In their aggregate, all the green plants now fix approximately 840 $\times 10^{12}$ kilowatt hours of solar energy per year in the form of biomass. This is more than 10 times the amount of energy that all of humankind uses annually, even with its most extravagant technologies. Who can doubt that this continuous process of accumulation of organic matter and energy will eventually affect both the surface of the Earth and various forms of life. Furthermore, Lovelock himself points out that the process of change may pick up speed and complexity as a result of human interventions, and he appropriately quotes me in stating that, on a local level, profound co-evolutionary changes have occurred in certain terrestrial environments and in their biological systems during historical times.

In the last chapter of his book, Lovelock has the courage to explore the relevance of the Gaian hypothesis to the effects of human interventions into nature. I wish I had space to explain why I agree with him when he states that environmentalists often shoot at wrong targets because the resiliency of the Earth, considered as an organism, probably makes ecosystems more resistant to pollution than commonly believed. As I cannot discuss these problems here, I shall conclude by expressing my wish that in the next edition of his book, Lovelock emphasises not only the homeostatic aspects of the Gaia hypothesis but also its creative aspects. This would be in the spirit of his statement that the Gaia concept is an alternative to the "depressing picture of our planet as a demented spaceship, forever travelling, driverless and purposeless, around an inner circle of the sun". □

René Dubos is Professor Emeritus at the Rockefeller University, New York.

Pollution in perspective

Kenneth Mellanby

A Perspective of Environmental Pollution. By M. W. Holdgate. Pp. 278. (Cambridge University Press; London, New York and Melbourne, 1979.) £15.

SOME ten years ago I was invited by a British publisher to write a book with the title *Pollution in Perspective*. This was to have been a 'popular' book in which I would try to distinguish between real, damaging pollution and trivial environmental contamination which, though harmless, nevertheless caused so much public concern. Being an idle fellow I procrastinated, and my text is still mainly in my imagination. Therefore, when I saw Dr Martin Holdgate's new book, with so similar a title, my emotions were mixed. On the one hand I felt guilt for not having completed my own book on such an obviously important subject, and on the other hand I experienced relief that someone else had done the job so competently.

For there can be no doubt that Dr Holdgate has produced an excellent book. Although he modestly denies having given his subject complete coverage, I cannot find any significant gaps. All major topics in the field of pollution are dealt with clearly, scientifically and with a full understanding of the facts, where these are easily available, and also the lacunae in our knowledge are always mentioned.

The book starts by placing pollution in its ecological context, and then goes on to define pollution and classify pollutants. A very important chapter is "The significance of pathways" which deals with the way substances are dispersed in the air, in fresh water and in the sea. The significance, sometimes overestimated, of the transmission of toxic substances through food chains is assessed. The great value of the book is that it constantly relates the concentrations of specific pollutants in the environment to the real effects on man or other targets. It thus shows how standards for pollution control may be scientifically determined, and how monitoring may be used to determine dangers and enforce controls. The costs of pollution control, and the way these may — or may not — be balanced against benefits are discussed. The international problems caused when pollutants cross frontiers are described.

The general theme is admirably stated in the book's last paragraph:

> "Finally, there are grounds for optimism. There appears to be no reason why pollution should be the doom of mankind or the destroyer of the ecological balance of the world. But pollution control is an essential element in sound environmental management. The higher the standards of environmental quality and safety we demand, the more effort we shall have to make, and the more it will cost. It is a matter of wisdom and judgement how much risk is acceptable and how high environmental quality should stand amid the other goals that compete for the resources of society".

No doubt there are those who will accuse Holdgate of "complacency" for not preaching doom and gloom, but thoughtful readers will welcome his clear assessment of the national and global situation. He shows that if mankind is destroyed by pollution, it will be our own fault.

Within its limits, which I shall discuss later, this book deserves nothing but praise. It is well written, and reasonably free from jargon. It will be a godsend to lecturers in universities, polytechnics and colleges of higher education who have to give courses on pollution or pollution control. Most of them could improve their current lectures simply by reading aloud from Holdgate's text.

My only criticism of the information given is that it is not always up to date. Dr Holdgate is a senior civil servant, with the characteristic respect for his sources, particularly where these are such things as the prestigious reports of the Royal Commission on Environmental Pollution. He reproduces (as others have done) a number of their admirable diagrams. For instance, from their first (1971) report we have graphs showing the amounts of smoke and sulphur dioxide in Britain's air at different dates. These figures show the remarkable improvement in air quality between 1950 and 1968 or 1969. For smoke, we are even given the "projection" for the year 1975, four years before this book appeared. As Dr Holdgate is Director General of Research in the Department of the Environment, where there are many junior scientists who have up-to-date figures for the period since the Royal Commission reported readily available, it is a pity that the diagrams were not redrawn and updated. This is not a trivial point, for the result would have reinforced many of the book's arguments, for the figures would have shown that there has been a further, substantial, improvement in air and water quality right up to the present day.

Notwithstanding this minor fault, this is a book which deserves a wide circulation. However, I fear that its sales will be reduced by the way it has been presented by the publishers, who have not served their author well. The text is set in reasonably clear type, but it is much too small to make reading a pleasure. The sections all bear unnecessary numbers (for example, 2.5.1 Pollution in the air) which may make prospective buyers who run through the pages in a book shop think that they are being offered a government report, and so they will be put off. Finally, the price of £15 for 278 pages (with no expensive plates) will not deter the lecturer who has to deliver a course on the subject, but his students will not be able to afford their own copies. This may, of course, be welcomed by some teachers, who will be able to keep up the pretence

that their oratory arises form their own original deliberations. The book is also too costly to circulate widely among concerned members of the public who need the information to allow them to make correct decisions on environmental problems. Also, though well written, the style is of the good scientific paper, which may present difficulties to those used to lighter literature.

So, although Dr Martin Holdgate's *A Perspective of Environmental Pollution* is a fine book, valuable to the élite, it may not reach the mass audience envisaged by the publisher who wanted me to write *Pollution in Perspective*. So I fear that Dr Holdgate may not have let me off the hook, and that I may still have to write my own book. If I do, I, like all future writers on this topic, will have to thank him for marshalling so much information so admirably, and for reducing our work so considerably. □

Kenneth Mellanby is Editor of the international journal Environmental Pollution.

Pragmatic humanism

Stephen H. Schneider

The Climate Mandate. By W. Roberts and H. Lansford, Pp. 197. (Freeman: San Francisco and Reading, 1979.) Hardback £6.70; paperback £3.30.

In the past five years at least twice this number of popular books on climate has crossed my desk. Some promise ice (for example, *The Cooling*), others fire (for example, *Hothouse Earth*) and one even has the title *Ice or Fire*? Among these offerings one of the most recent, *The Climate Mandate*, is one of the best. Its authors are Walter Orr Roberts, former Director of the National Center for Atmospheric Research (NCAR) and Henry Lansford, a science writer and former Informaion Officer at NCAR. Dr Roberts is presently Director of the Program in Food, Climate and the World's Future of the Aspen Institute for Humanistic Studies. His concerns with humanity are well reflected in *The Climate Mandate*.

The team of a scientist/humanist and a science-writer has resulted in a very readable (and mercifully short — only 197 pages) selection of topics related to climate and climatic change. We are spared the uncaveated railings and pet theories of some climatic prognosticators that climatic doom is nigh as well as the bland assurance of some ostrich-like professional meteorologists that there is no climate problem worthy of public note, let alone a dozen books.

Of course, in 197 pages one cannot fully lay out the range and depth of all phases of the climate problem. *The Climate Mandate* will thus likely be only a partial help to those looking for a non-specialist textbook on climatic change to supplement, say, a beginning level college course in geography. On the other hand, it is much better than most of its competitors in confronting directly the place of climate and climatic variability in the world food picture. In fact, the book is organised to build up to the last chapter ("Defining the Problem — Malthusian Pessimists, Social Idealists, and Technological Optimists") in which the authors offer scenarios for world development within the demanding constraints of population growth, environmental stress, ideological differences, economic inequities and, of course, fluctuating climate. The scenarios comprise "the climate mandate".

To back up their solutions to the world predicament, there are chapters which review briefly climate history; the physical factors influencing climate; the climate theories of various scientists; the difficulties of climate forecasting; the respective roles of climate; technologies and infrastructure in crop productivity; and a cautious description of some weather modification operations in the US. If one extrapolates slightly from their carefully hedged remarks on weather modification, they seem to suggest that more dollars have flowed from granting agencies to weather modifiers than from seeded clouds to water users. They caution that such technological fixes are not panaceas, an apt thought in which to transition to the final chapter, where the food/population/energy/environment/climate problem (that is, the human predicament) is addressed.

The human predicament had been addressed often in the two hundred years since Malthus, and Roberts and Lansford bring it up to date with an overview of three modern contrasting views. First, there are the "Malthusian pessimists", personified by ecologist Garrett Hardin, who counsel "lifeboat ethics", whereby rich countries like the US are advised to abandon hope of feeding chronically poor countries with high population growth rates. The "technological optimists", on the other hand, believe that modern energy and capital-intensive farming can easily accommodate the foreseeable growth of world population — if only Third World governments would provide markets for farmers to sell their produce and incentives for corporations to transfer their technologies. Finally there are the "social idealists", who fear that transfer to the poor of modern large-scale technologies is another form of imperialism, as it makes the developing countries dependent on products developed and produced abroad. They counsel the overthrow of the "elites" who dominate Third World governments and the establishment of locally self-reliant, labour-intensive production systems.

Roberts and Lansford believe, as do I, that it is simplistic to view the human predicament simply as a problem of monolithic cause ("the danger lies in the conviction that a single aspect of the problem is so overriding that attempts to deal with any others are simply a waste of time and money"). They propose, instead, an approach they call "pragmatic humanism", in which we are to accept the "premise that many human lives can be saved and much misery avoided by a diversity of efforts". Technology or the Green Revolution are legitimate tools in this "whole-system approach", but their applications must meet local needs. The authors then offer "scenarios" which comprise "the climate mandate". The first element is to establish a "world food emergency reserve" and distribution system to mitigate the effects of local climatic variability. (This reviewer could hardly disagree without revoking his own writings.) Next is the need to develop agricultural self-reliance in developing nations — with US and USSR joint-help. The key to success is flexibility and cooperation on all sides. Specific examples of how to bring this about, however, are few. According to their scenarios the Earth could "have the ability to produce adequate food for a doubled or tripled world population", but they admit (and unfortunately this is the only reference to this crucial issue) that "the ecological implications have not been examined in any comprehensive way".

The authors conclude optimistically, despite the litany of obstacles they properly point out, that "if we heed the climate mandate, and if we accept the fact that the Earth's people are bound together by mutual needs and expectations that must transcend our rivalries and contests, humanity should be able not only to survive but to prevail over the hunger and starvation that have threatened so many people for so many centuries.".

The Climate Mandate is a compassionate book; it reflects the authors' own humanity. Its hopes and sentiments are appealing. But if past experiences with global cooperation remain our only reference, I would continue to rate the prospects as "technologically optimistic, but politically bleak". □

Stephen H. Schneider is a climatologist at the National Center for Atmospheric Research, Boulder, Colorado, and Editor of the journal Climate Change.

H. Stephan, G. Baron, W. Schwerdtfeger

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Nuclear detective story

J.H. Fremlin

Nuclear Disaster in the Urals. By Zhores A. Medvedev. Pp.214. (W..W. Norton: New York; Angus and Robertson: London, 1979.) $12.95; £5.95.

DR MEDVEDEV has spent much time in studying widespread but indirect information about a very serious nuclear accident which occurred in the Urals, seemingly around 1957. His book begins with an extensive account of the hard and ingenious detective work which was needed to extract information from seemingly irrelevant papers on radioecology published in Russia, often years after the event. He gives an impressive picture both of the all-pervasive thoroughness of the censorship and at the same time of the number of points which nevertheless slipped through, and from which he has built up a convincing picture of serious contamination over a large area on the eastern side of the southern Urals where the original Soviet military nuclear industry was situated.

Dr Medvedev seems unnecessarily anxious to establish his excellent case for the reality of the disaster, and his arguments are not always individually convincing. For example, he says that papers written during the following few years on the radioecology of the region purported to describe the take-up of radioactive material, especially ^{90}Sr (strontium-90), by plants and animals and the food chains connecting these. To do this properly, he claims that less than 10% of the animals concerned could have been trapped or shot without disturbing the ecology which it was desired to investigate. This is hardly relevant, at least in the short term, for deer which are at the end of a chain (assuming that human hunters are not to be sampled). Using this figure of 10%, he deduces that the area contaminated must have been at least ten times the area found by dividing the number of deer shot, by the average population density. It could however, have been intended to shoot 100% of the larger contaminated ungulates, to prevent them from migrating to regions where they might later be shot and eaten. In this case one would deduce a maximum area ten-times-smaller than Medvedev does.

The book has clearly been written rather hurriedly, leading to some errors. For example, on page 173, he states that ß-particles can penetrate 2 — 8 cm into living tissue and he (or the translator) has missed a misprint of protons for photons. More seriously, on page 74, he defines the permissible dose of radiation as being the amount that is assumed not to cause harmful effects, and on page 21 says that the next generation will suffer severely if they are born from parents with ^{90}Sr in their bones. This and its daughter product ^{90}Y (yttrium-90) are both pure β-emitters with a range of only a few millimetres in tissue.

In spite of these minor errors, I accept without reservation that a major accident must have happened around 1957 which contaminated probably tens and conceivably hundreds of square kilometres — to a level of 1000-4000 Ci/km^2. For the reason given above, I do not accept the numerical estimate of 400 square kilometres of heavy contamination, but there seems no doubt that Dr Medvedev is right in describing the event as a major disaster, and that this was connected, like our Windscale reactor fire, with the military production of plutonium for bombs.

The final sixth of the book is speculative. The censorship has successfully hidden the cause of contamination. The US intelligence services may know the truth, but the thoroughly "sanitised" documents extracted from the CIA do not divulge it. Accordingly Dr Medvedev has had to rely on hearsay. Early guesses by local but uninformed people included an accidental bomb explosion and an escape of material from a runaway reactor. A third guess is that the wastes left after extraction of plutonium and unsatisfactorily stored, exploded. At first sight either of the first two seems possible and the third seems impossible on thermodynamic grounds, for the same reason that you cannot make a good fire with the ashes of an earlier one. Nevertheles, this is the theory that Medvedev espouses, and as his arguments are plausible they need to be examined.

The major positive argument is the size of the area affected. In each square kilometre the ^{90}Sr observed was of the order of that produced by the fission of a kilogram of uranium or plutonium. By 1957 the accumulated wastes could have sufficed to cover 400 km^2 if an adequate distribution mechanism existed. So could a 'dirty' H-bomb, with no distribution problem; but a fire in a runaway reactor could hardly do so. The actual area covered by heavy contamination is therefore important.

The second argument is the low ratio of ^{137}Cs to ^{90}Sr which varied from 1:8 (in a lake) to 1:1000 in soil, instead of the roughly 1:1 expected from fission. Dr Medvedev suggests that most of the ^{137}Cs had been removed from the wastes for industrial or medical use. The large difference between lake and surface soil, however, looks much more like the result of leaching away of the mobile caesium in the soil leaving the less mobile strontium near the surface.

The chief argument against the waste-explosion theory is the impossibility of producing an explosion which would pulverise the (non-volatile) material to the 1—10 μm sized particles needed to spread it over hundreds of square kilometres. Such an explosion would have to be of the same order as the Bikini H-bomb test and is not consistent with the type of steam explosion that could arise due to water running into radioactively heated waste. This could throw a lot of material for hundreds of metres but could not produce the fine pulverisation of the whole mass of material needed for its uniform distribution.

Dr Medvedev's alternative source of energy lies in the plutonium with which the wastes are invariably contaminated. At the Hanford works in Richland, Washington (one of the largest atomic plants in the US) some 100 kg of plutonium was concentrated over a decade, by a process equivalent to chromatography, in the topmost layer of mud in the trenches into which liquid wastes were run. This process might indeed eventually produce a critical concentration and this could support a chain reaction. This would, however, necessarily start slowly with a "delayed-critical" concentration in wet material, as the plutonium could not be chromatographically concentrated in the dry. This reaction would probably raise the temperature and reduce reactivity to equilibrium values at which plutonium was burnt as fast as it was added. At most it could raise the temperature only so fast (the delayed doubling time being several seconds) as again to produce a steam explosion. It is difficult to see how this could reach the effect of even a ton of TNT and quite impossible to see how it could reach the megaton scale required to contaminate hundreds of square kilometres.

Although the visible flash lighting up the sky reported by the CIA in 1955 (page 195) is consistent with an accidental H-bomb explosion, I do not think this the only possible explanation of the facts. I am unconvinced by Dr Medvedev's ecological arguments that the area involved was 400 km^2, and a very respectable smaller area could have been contaminated to the level observed by an uncontrolled reactor fire in which a large part of the uranium fuel was burnt and dispersed with the smoke of burning carbon moderator. Dr Medvedev realises himself that there are difficulties with any of the mechanisms proposed for a waste explosion. In his own words (page 163), "Truly, scientific imagination (or a capacity for 'science fiction' if you will) is needed to construct hypotheses about the exact causes of the explosion". I can only agree. But I hope a lot of people will read the book and see whether they agree too. (I congratulate the translator.) □

J.H. Fremlin is Professor of Applied Radioactivity at the University of Brimingham, UK.

Unorthodox epidemiology

D.A.J. Tyrrell

Diseases from Space. By Fred Hoyle and Chandra Wickramasinghe. Pp.196. (Dent: London, 1979.) £6.95.

THIS is an unusual book. It is well produced and easy to read, being written in a narrative and popular style; but its six chapters and three appendices are apparently intended to be a serious presentation of a theory put forward by the authors. The subject matter ranges widely. They start with cosmology and their interest in the organic molecules to be found in interstellar space. They suggest that life may have evolved in cometary bodies composed of organic chemicals, which would have had an inner zone protected from radiation and warmed to about 10°C by internal chemical reactions. In this "pool", primitive life-forms like bacteria and viruses evolved and then, when the comet passed near the Earth, were shed into space as micrometeorites, protected from ultraviolet radiation by a coating and sufficiently small not to be overheated on entering the Earth's atmosphere. They then sank towards the Earth, were carried on down to its surface by storms, and thus initiated biological life in the primeval Earth. They further assert that the process continues and that clouds of viruses, particularly those of influenza and the common cold, rain down upon the Earth and cause epidemics of these diseases; and that epidemics of smallpox, and of bacterial diseases like cholera and plague, may occur in the same way.

The authors have obviously read widely and thought deeply, and I am unable to express a useful opinion on their theory of the origin of life. However, as they extend the theory to explain epidemics, this can be examined in relation to present knowledge of infectious diseases. For instance, they argue that influenza viruses descend from space and are breathed in by birds, animals and man, and that the transmission from person to person or animal to animal is unimportant. They do not explain how an organism which must have produced untold billions of particles in a cometary puddle in space (and which they suggest may have arrived on Earth inside a bacterium) was also exquisitely adapted to replicate in avian and mammalian cells. The influenza virus has specific functioning elements by which it penetrates membranes, involves the cell nucleus, is replicated in ribosomes and forms new particles by inserting viral protein into the cell membrane which then bulges out and envolopes the virus nucleic acid and other peptides lying in the cytoplasm beneath. In Earth laboratories many common cold viruses, rhinoviruses and coronaviruses multiply only in human cells, and only some such cells — in what sort of cells do they replicate in space? The influenza viruses found around 1930 have almost exactly the same internal peptides as those that circulate now — does all influenza come from one cloud of meteorites?

A key fact of their argument is that they have looked at published literature, investigated outbreaks of influenza in schools and found no evidence that influenza spreads from person to person. They state that if influenza did spread in this way it would be distributed in the children in a school by a random process. They show that it is not random — at Eton some houses were heavily affected and others virtually free, as judged by clinical diagnosis — so they prefer the earlier theory that influenza is due to a "miasma". It is stimulating to have current thinking challenged in this way, but I doubt if their theory provides a better model to explain known facts than do our present concepts. There is evidence that different individuals shed varying amounts of virus, so in small groups they would be expected to infect at different rates — and I think the spread of the recent H1N1 virus from China across the USSR, and to Hong Kong and across the world, is better explained by spread from person to person than by viruses descending from space first in one area and then in the next. I would expect an influenza virus coming from space to appear over a very wide area if it really has to sediment slowly and negotiate the jet streams as the authors describe, even if it does survive desiccation and ultraviolet radiation.

I cannot agree with the theme of the book, which smacks more of science fiction than of science, but I think it is good that it has appeared. I would hate to think that unorthodox views could be suppressed, or that authors could never be published if editors or reviewers did not like their work. □

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US politics and the test ban

Edward Bullard

Blowing on the Wind: The Nuclear Test Ban Debate, 1954-60. By Robert A. Devine. (Clarendon/Oxford University Press: New York and Oxford, 1978.) £7.95.

THIS book is a detailed account of the reactions of US politicians, scientists and the public to the attempts to stop tests of nuclear weapons. It is confined almost entirely to the time when Eisenhower was President (1952-60) and is a political and sociological study rather than a technical discussion. Technical questions are mostly dismissed with the statement that some scientists said one thing and others said the opposite.

To have the events, and the views of the people concerned, set out in chronological order is useful. There are some well-documented judgements that run counter to widely held beliefs; for example John Foster Dulles appears as a strong and consistent supporter of a test-ban.

Useful as it may be, the book makes dull reading. This is, in part, due to the almost exclusive concentration on US views; negotiations require at least two parties and the interest lies largely in the contrast of aims and methods of the two sides. The extent to which this book ignores all non-US participation is lamentable. The only references in the bibliography to publications by non-American authors are to Khrushchev's memoirs (transcribed and edited in the US), *Sakharov Speaks* (also a US publication) and Harold Macmillan's memoirs. The last of these is the only item in the bibliography that is not published in the US. It is, of course, difficult to understand the motivations and internal struggles of a closed society, such as the USSR; it is, however, important to make the effort. The first route to understanding is the study of official pronouncements and propaganda. If propaganda loses touch with the real world and with real intentions, it loses plausibility and becomes unconvincing to those it is intended to influence or deceive. For example, it was possible to follow the German retreat across Russia in 1944 by noting the westward progression of the locations of alleged German victories. For the Russian views on the test ban there is much more evidence than propaganda.

The 1958 'expert' discussions hold a special place as the first discussions with the Russians in which there was any real meeting of minds. Stalin had been dead for 5 years and Khrushchev had consolidated his power. There was a new situation in which Russian good faith and intentions had to be assessed anew. These matters are inadequately dealt with by the author who seems to have read only the final agreed report and not the transcripts of the day by day discussions, which are much more instructive.

The 1958 meetings were also of interest from another point of view. The American delegation consisted of a mixture of Hawks

and Doves who had not reached agreement on a 'party line' before setting out for Geneva. The reason for this probably lay in Eisenhower's belief in consensus and dislike of controversy and of having to decide between contesting parties. The meetings of the western delegates were often difficult. Most of those present were interested in assessing Russian intentions and motives, but the Hawks were primarily concerned to prevent any kind of agreement wiht the USSR. They even maintained, for a while, that atmospheric tests could not be sufficiently monitored because the wind might blow round and round in circles and never carry the radioactive debris out of Russia. By confining himself to published sources and Presidential papers the author has missed the details of these interesting controversies. He seems not to have discussed the negotiations with any of the participants.

The breakdown of the negotiations for a complete test-ban came in the political talks late in 1958. The US withdrew its support for the conclusions of the 'experts' concerning underground tests, introduced hastily analysed data from recent tests and declared that the technical matters must be discussed *de novo*. This interesting situation is very inadequately discussed and no references are given to Russian views; for example, to the detailed discussions by Riznichenko.

Throughout the book the discussion of technical matters is marred by an apparent lack of interest in the meaning of numbers. For example, on page 103 it is stated that Willard Libby calculated the amount of Sr-90 in the atmosphere to be 24 megatons. It is doubtful if every reader will understand this oracular statement. Much more confusing are the two values given for the amount of Sr-90 in the soil of the US in 1958 (pages 263 and 272); these differ by a factor of a million. The larger of them is a factor of 1000 less than that given by the AEC for 1961 in *Effects of Nuclear Weapons*. This muddle may be connected with the author's belief that m is the abbreviation for micro.

Perhaps I have been unduly severe, the book is about American politics and my complaints are that it is not about other aspects of the subject. It does unearth some fascinating things, such as a remark made in 1958 by a member of the President's Scientific Advisory Committee. He (who was he?) is said to have said: "These men who don't want a test moratorium are like a kid you are trying to put to bed. First he wants a drink of water and then he wants to go to the bathroom, but what he really wants is not to go to bed". □

Sir Edward Bullard is an Emeritus Professor of Geophysics of the University of Cambridge, UK, and of the University of California at San Diego. He attended the 1958 Geneva discussions on the possibility of a test-ban treaty.

Commitment to logic

Aaron Novick

Origins of Molecular Biology: A Tribute to Jacques Monod. Edited By A. Lwoff and A. Ullmann. Pp.246. (Academic: New York and London, 1979). $17.50; £11.35.

MOLECULAR BIOLOGY had its origins in several centres which served as gathering places for people from around the world. In the very beginning, it was Cold Spring Harbor in New York and soon after the Institut Pasteur in Paris and the MRC Laboratory in Cambridge, among others. These places developed for a variety of reasons not the least important of which was the presence of some of the leading figures in this new field. At the Institut Pasteur it was André Lwoff, Jacques Monod and Francois Jacob. Jacques Monod died in 1976 and a number of his colleagues and disciples have contributed to this *Tribute*, a volume which describes from a number of points of view an exceptional person who played one of the major roles in the development of our present understanding of the control of gene expression. From its title, it seems to be a companion volume to *Phage and the Origins of Molecular Biology*, edited by J. Cairns, G.S. Stent and J.D. Watson (Cold Spring Harbour Laboratory, 1966), a volume dedicated to Max Delbruck on his sixtieth birthday.

The personal accounts in volumes such as these provide descriptions of important and fascinating details in the development of scientific ideas never reported in the usual forms of publication. Because of its memorial character, the present volume includes many personal details in its descriptions of a man of great spirit whose life outside the lab as well as in it was of heroic scale, whose friends included scientists and public figures throughout the world.

Of particular interest is the collection of personal statements from most of the people involved in the series of discoveries which culminated in the giant step represented by the Jacob-Monod model for gene expression and regulation. The reports provide a fine illustration of the development of a great scientific idea, but one must recognise that the picture is limited by the modesty of some of the participants in this memorial volume. André Lwoff and Francois Jacob were no less important both to the exciting and creative spirit at the Pasteur and for their own remarkable scientific contributions. As a history, the volume does not make it adequately clear that the critical experiments which showed that control of the *Lac* gene occurs through action of an endogenous repressor could be done only because of Jacob's timely development of the necessary genetic technology.

It is also fascinating to follow the development of Monod as a scientist. Here is a man for whom science was a great personal adventure and a means of personal expression, yet a man fervently dedicated to logic and reason. He exulted in the power of deduction and was known to shout after an observation verified one of his deductions that this was again proof that Descartes was right and Kant wrong. Despite his commitment to logic, he had a remarkably successful intuition for how things must be, and he could present these pictures with great force. Even when he was later proved wrong, the vehemence and sharpness of his argument expedited the clarification of a problem.

Some of the authors also note his audacity. He never limited himself to the disciplines of his education nor did he ever restrict himself to minor objectives. In his crowning work, the development of the allostery model for regulatory proteins, despite being trained as a zoologist he used modern physical chemical ideas with no inhibitions to formulate a remarkably original and innovative theory. "It is no longer a question of right or wrong", one of my colleagues in the field remarked. "The Monod theory has changed the style of thinking among the biochemists."

An impressive feature of the volume is the honesty of some of the reports which describe the personal faults as well as the greatness of Monod. His enthusiasm and pursuit of fundamental problems inspired the people he worked with. His candour came as an exciting surprise when it was first encountered by his disciples. This lack of stuffiness along with his generosity and the confidence he placed in his students created love and great loyalty. But he also had personal faults, and these too are reported. He could be vain and arrogant in a pointless way. Surely it is because they held him in such high regard that his colleagues were free to describe these faults so honestly.

As occasional allusions in the volume indicate, Monod's life outside of science was also remarkable. He was aware, wise and, when he felt it necessary, active in the world of politics. During the hysteria in the United States in the early 1950s, when foreign visitors were required to sign non-communist affidavits, the *Bulletin of the Atomic Scientists* published statements by a number of European scientists; I recall that by far the best of these was the one by Monod, who with characteristic perception saw the larger implications of US policy. Again, on the occasion of the Nobel Prize, rather than basking in the adoration and pride the French have for their Nobel Prize winners, he took advantage of the public attention to improve the support of science in France by making a strong public statement severely critical of his government's science policy,

a statement which apparently infuriated DeGaulle.

Some contributors make reference to Monod's service during World War II in the Resistance and later in the re-established French Army, but the volume's guidelines prevented fuller description of these parts of his life, for the same reasons the authors say little about Monod's significance as a public philosopher, illustrated by his book, *Chance and Necessity*, which was a sensational bestseller in France.

The essays are generally of exceptionally high quality, and they will bring back memories of great times for the large number of scientists throughout the world who have spent time with Jacques Monod and his colleagues in those laboratories built originally for Louis Pasteur. It will also bring the pleasure of enlightenment to many others who were not directly involved. In addition, for those totally outside of molecular biology, it is a worthy addition to the developing literature on the origins of this field which has so revolutionised biology. And this appearance of a memorial volume ought to stimulate interst in a more complete biography of this impressive man whose life touched so many of the important developments of the middle decades of this century. □

Aaron Novick is Professor at the Institute of Molecular Biology at the University of Oregon. He spent 1953-54, 1962-63 and 1967-68 in Jacob and Monod's laboratories.

Challenge to promissory materialism

Anthony Flew

The Human Mystery. By John C. Eccles. Pp. 255. (Springer International: Berlin, Heidelberg and New York, 1979.) DM34.

EVEN had Sir John Eccles not begun this his own first series of Gifford Lectures with quotations from "My great master, Sherrington" (page 2) any sympathetic reviewer would have been bound to refer to the fitting facts that it was just 40 years earlier, under the same foundation and in the same University of Edinburgh, that that "great master" himself gave the lectures which were later to become *Man on His Nature* (Cambridge, 1940). *The Human Mystery*, though thoroughly worthwhile, lacks the magic of that predecessor: how could it not? The present Eccles project, of which we have here only the first and probably less distinctive half, is strictly in accordance within the terms of Lord Gifford's will: ". . . to promote and diffuse the study of Natural Theology in the widest sense of that term — in other words the knowledge of God".

The aim is to mount a challenge to what, following his friend Sir Karl Popper, he calls "promissory materialism", a challenge along the entire front but with the main weight concentrated in the sector of his own lifelong concentration, the human brain. Promissory materialism is defined as the "claim that science is so powerful and pervasive that in the not too distant future it will provide an explanation in principle of all phenomena in the world of nature including man, even human consciousness in all its manifestations" (page 7). So, after a first introductory lecture, Eccles proceeds through the "Origin and Evolution of the Universe", the "Planetary System and Planet Earth", the "Origin of Life and Biological Evolution", to "Human Evolution: The Story of Cerebral Development". It is in the second half that we come to the real speciality of the house: "Cultural Evolution with Language and Values: The Human Person"; "The Creation of a Self"; "Structure of the Neocortex: Conscious Perception"; "Learning and Memory"; and "The Mind-Brain Problem".

To the scientific layman the story which Eccles tells in his first half is fascinating, though here and elsewhere to follow him closely requires frequent consultation of those handy Penguin dictionaries of the sciences: if he has succeeded in his attempt "to present scientific material . . . with a minimum of technicality", then one must pray to be spared the failures.

The Gifford aspect appears at once in "the Big Bang story as suggesting the operation of a supernatural Creator" (page 15); although it is curious that Eccles mentions only in an aside the notion of a Creator upon whom — whether or not, He, or She, 'in the beginning' also started the Universe off — it continues to be always absolutely dependent. After all, it was precisely and only continuous creation in this theological sense which St Thomas insisted could be known on the basis of natural reason, without benefit of revelation.

Two other Gifford points stressed in the first half are the unpredictable chanciness of the emergence of life, and of the actual course of biological evolution; and the fact that the whole cosmological story would have had to be very much as it has been if beings capable of knowing that story were going to become part of it. About the former Eccles maintain, and surely he is right, that "There is no predictable line of evolutionary development" (page 49). It seems to be as true of the evolutionary biologist as of the human historian that most of his most important explanations are of how something was possible, rather than of why it was inevitable. No doubt other readers will share my own pleasure in two pieces of Eccles mischief: the suggestions that the most primitive mammals "brought about the dinosaurs' downfall by preying on their eggs" (page 58); and — of "the attempt to communicate with super-intelligent humanoids in our galactic system" — that it is "highly likely that this romantic project will be killed by boredom" (page 73).

In wondering what to make of the second of the two points stressed in the first half we must give some weight to the point, which Eccles does not notice, that these conclusions are derived only for a Universe obeying the same fundamental laws of nature. The argument is that "If we economise and have the Big Bang producing the mass for one galaxy of 10^{11} stars, which is still an immense universe", then, given what we now know of the fundamental laws of the actual Universe, "the time from Big Bang to Big Crunch is reduced to one year" (page 28). But a Creator, having it in mind to furnish his creation with potential knowers, would not, presumably, be restricted either to or by any actual or possible natural laws.

In his rather longer second half Eccles is at least equally fascinating. In lecture 6 he begins by contending, as is surely correct, that "cultural evolution is exclusively human" (page 98). This and much of the later discussion is presented in terms of Popper's distinction between "three Worlds: World 1, the matter-energy of the cosmos; World 2 of all subjective experiences; and World 3, which is the cultural heritage of mankind" (page 98). The reader needs to be aware always, as their spokespersons so often are not, that the second and third of these can be identified only as aspects of the first. We have, that is to say, no knowledge of any kind of consciousness other than the consciousness of organisms; and perhaps we cannot even give sense to talk of any consciousness that is not thus rooted in the matter-energy of World 1. The whole cultural achievement of World 3 is likewise embodied in, and inseparable from, such incontestably World 1 objects as buildings, books and computer tapes.

In the rest of this lecture Eccles considers the enormous importance of language and — what could not exist without language — the self-consciousness which is so much more than mere consciousness. As one expression of his constant and most welcome concern to resist the *1984* which is "engulfing more and more of our planet" (page 237), and after crediting to the Sumerians the two incomparably important inventions of both writing and the wheel, Eccles remarks that for most of its history Sumer "was an amazingly open and tolerant society" (page 114); though it does not occur to him that those market

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interests which he was earlier inclined to chide might have been one necessary condition of this tolerance (page 110).

Lecture 7 examines "the role of World 3 in producing the next generation"; making much, but never too much, of the crucial fact that it is thanks to this that "The creation of a cultured person" can now "be accomplished in a few years", whereas "it took at least 50,000 years to create a high civilisation" (page 123). This chapter contains one section which will especially delight any philosopher who has ever faulted Hume's *Treatise of Human Nature* for its "paralytic's eye view of causation": Eccles tells of experiments with kittens in gondolas which show that exploratory learning will not take place without active movements by the learner (page 132).

Lecture 8 on "Conscious Perception" constitutes necessary preparation. In lecture 9 on "Learning and Memory" the thesis is that memory is essential to anything deserving to be called knowledge: "Without memory there could not be a knowing of existence" (page 180). In lecture 10 Eccles wants to construe mind and brain as "independent entities, the brain being in World 1 and the mind in World 2 (Fig. 6-1)", and to urge that these are both entities which "somehow interact, as illustrated by the arrows in Figure 10-2" (page 212).

But this, as the rally-drivers say, is going a bit quick. For all our ordinary talk about minds, ordinarily understood, is just talk about certain sorts of things which some creatures of flesh and blood can and do do or suffer. To say that, for instance, she has a first-class mind just is to say that she is capable of operating at that high academic level. So if anyone wants to hypothesise some independent entities to be called minds, he has first to provide some account of how such entities are to be identified and individuated. For minds in the common everyday sense of the word are not what is required: they are not, that is to say (logical) substances — things which, like chairs and tables and unlike grins and tempers, could significantly be said to exist separately and in their own right. To speak of her first-class mind surviving her death and cremation would, in the everyday sense of 'mind', be about as sensible as speaking of the grin on the face of Alice's Cheshire Cat outlasting the cat of whose face it was a configuration. On the other hand, much of the history of philosophy since Descartes can be read as showing how difficult if not impossible it must be to think up a notion of minds as immaterial substances; which could be identified as subjects of discourse, re-identified through time, and individuated one from another. □

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Precious cult of scientific knowledge

Martin Pollock

Paradoxes of Progress. By Gunther S. Stent. Pp. 231. (Freeman: San Francisco and Reading, UK, 1978). Hardback £8; paperback £3.90.

THIS is a collection of articles on modern science, already published over the past ten years or so and now strung together, a little forcedly perhaps, under a title which can serve a multitude of themes. It is therefore, inevitably, somewhat of a ramble. The common thread is Professor Stent's own idea of what is happening to our precious cult of scientific knowledge, where it is going and what its principles mean, or should mean, for us. His opinions are worth studying, though cramped by a rather awkward style of writing and even if — or especially if — one does not agree with them. The problems he raises and the questions he asks are those which most scientists do not even like to think about, let alone try to answer.

All the essays are worth reading in their own right, although two of them — a review of Jim Watson's *Double Helix* and an analysis of "Prematurity and Uniqueness in Scientific Discovery" — stand rather apart from the rest, being of borderline relevance to the central issue. Three are of outstanding interest. These are: (1) "The Dilemma of Science and Morals" which discusses the many desperate problems of how our continuously expanding knowledge should be applied practically and includes a brief but fascinating comparison between the science and technology of classical China and western scientific philosophy; (2) "Limits to the Scientific Understanding of Man", being essentially a clear-cut criticism of positivism and its later manifestations in the form of simplistic acceptance of a reality that is "built on cumulative experience" alone and is now referred to as "Scientism"; and (3) "The Decadence of Scientism" ('Decline' might have been a more appropriate word), which discusses a neo-Kantian basis for understanding and a practical morality that recognises the impossibility of true objectivity or value-free conclusions, and involves adopting what is now often referred to as a "Structuralist" and anti-beviourist approach to thinking and acting; this itself depends on analysis of "deep structures" beneath the superficialities to which alone our senses are directly responsive.

According to Stent, science, as we now know it, is on the way out. The spirit of "Faustian man" who believes in "progress" and outward striving (defined as the "will to power") is on the wane and yielding diminishing returns; it is anyway self-limiting because it seeks, and to a large extent obtains, a sense of security which quenches the thrust for more power. Faustian man, born in the ages of enlightenment of the seventeenth and eighteenth centuries and reaching maturity in the nineteenth where the solution to all problems was viewed in the context of a continuously progressive increase in knowledge and power over the outside world, is on his death bed. His spirit will be succeeded by something similar to the Polynesian-based dream of a life of ease but without grand ambitions, a rejection of the idea of "progress" and materialistic sophistications and an acceptance of the Zen principle that the recognition of subjective sensation is the only reality. The "noble savage" is not a fancy directed backwards to the distant past, but something to anticipate in the years to come. The different articles share a common theme by illustrating the problems and paradoxes which herald the end of our era. And the author, who has rightly emphasised the many analogies between science and the arts, believes that similar factors are involved in the decline of the arts which have now lost all style and discipline, become totally "freed" and will have to start again with a new impulse.

One of the first signs of the end was, according to Stent, the "beatnik" movement in Berkeley of the late 1950s and (to a later and lesser extent) the hippy creed of the 1960s. Amongst scientists themselves, the relevant factors include a growing sense of the limitations to the objectivity of our observations and conclusions and increasing estrangement between our intuitive common sense and what we have to accept as most appropriate explanations; in particular, the principle of indeterminism in physics, which is equivalent to saying that like causes do not necessarily have like effect would seem to undermine the fundamentals of scientific explanation.

Stent also refers to a phenomenon described by Mandelbrot termed "second-stage indeterminism" where probably random events, such as "noise" in an organised system, theoretically able to be eliminated by a statistical approach, nevertheless seem on analysis for limited periods, to conform to certain laws of fluctuation (for example, in coin tossing experiments). This problem is the old one of how to decide when to introduce the concept of chance and what the idea of randomness really means — a dilemma which has faced scientists for very many years. To what extent, for instance, are the structural patterns we think we see around us simply illusions, based on too superficial and prematurely completed analysis of a series of events that require an impractically long period of study for

establishing their truly random nature?

One might go further on the basis of belief in subjective reality, and ask, even in the name of science, how many of the patterns we think exist in nature are only a reflection of pre-determined patterns in our brains. But we would still have to try and decide how our brain patterns appeared in the first instance.

Associated with the rather difficult idea of "second-stage indeterminism" is that of the "spontaneous activity of the system itself" which Stent also pursues. This is really rather frightening. Are we to suppose that every system in nature has some inherently endowed freedom of action which transcends natural laws and is thus immune to scientific study? If so, it might still be argued by Mandelbrot (if not by Stent) that this is no more than what most people ascribe to the human organism in the form of intrinsic freewill, upon which we all base our ordinary lives (*and* our science) whether we believe in it or not. And so we pass on to the equally difficult concept of *self* and one's own consciousness, forever a stumbling block for most philosophers. Even the most positive of positivists would surely have to admit that to use the machine itself for studying the machine is begging a shattering question and must involve some crucial assumptions, such as that the "thing in itself" is "truly" (whatever that may mean) what we perceive it to be, which few of us would be prepared to accept.

The final paradoxical problem raised concerns the moral dilemma of what should be the principles guiding our actions in science. And it looks here rather as if Stent is more than just vaguely sympathetic to the Taoism of ancient China which teaches that man is part of an inscrutable nature and must retreat from personal striving in order to attain inner fulfilment. This, of course, is the direct opposite of Western science where nature is *not* regarded as "inscrutable", but on the contrary responds strikingly to reason and has allowed us to exploit our environment with extravagant success — at least viewed from a strictly technological angle. With Tao, "instead of Truth and Justice", he writes, "Wisdom and Harmony would [then] become the primary values". And he quotes Joseph Needham: "with their appreciation of the relativism and subtlety and immensity of the universe, [the Chinese scientists] were groping after an Einsteinian world picture without having laid the foundations for a Newtonian one". Is the implication here that, in one sense at least, the Chinese have leap-frogged Western science and could soon be way ahead on the basis of a Tao-based structure for dealing with ourselves and the world around us?

Some recent advances in brain physiology are then described, in particular the fascinating work of Hubel and Wiesel on cells of the visual cortex. This emphasises the specific selective nature of



Gunther Stent in 1949

Photo: Cold Spring Harbor Laboratory

cell response to visual stimuli, indicating that patterns we "see" are made up of an integrated synthesis of the reactions of a composite of cells programmed by the combined effect of early environment and genetic predisposition to respond only to certain restricted types of input signal. This kind of selective mechanism of response to the immediate environment applies also to other types of (biochemical) cell reaction, such as enzyme induction. It is another aspect of the type of evidence that supports a "structuralist" approach to the mechanism of understanding, being closely related to Chomsky's ideas of "deep structures" in linguistics and has taken over, during the past 30 years or so, according to Stent, from the later stages of positivism expressed, for example, in "behaviourism" which is now largely discredited. And so, it is argued, we are back — or nearly so — to the original ideas of Kant in favour of "innate, *a priori* knowledge" (that is, *not* derived from experience) which could have evolved. Darwinistically, because it was the most suitable for survival at the time. And why not? If we cannot achieve absolute understanding, why not make do with the brain mechanism presumably evolved to supply the type of "explanation" most suitable for dealing with ourselves in the world as it is — or as it was!

Stent seems inclined to use a similar neo-Kantian method for postulating an "innate ethical deep structure", present in all human beings, which he recognises as not all that far removed from Kant's "categorical imperative", defined as an ethical principle so universally applicable that it has to be accepted as a natural law . . . . so obvious that it "goes without saying" — and thereby, one suspects, is different for every individual because nobody ever seems to have to formulate it in words.

One does not have to accept all or any of the implications offered in this book in order to benefit from thinking about them. The author is not very dogmatic, anyway, and only in his predictions about the end of our era in science does he attempt to make a firm case — which is, in fact, not very convincing. We may, perhaps be at a point of inflection in the development of science, but does it really amount to the end of an era? It is indeed, strange to view his arguments about the forthcoming "age of leisure" and a rejection of material efficiency, against a background of a world where nearly 50% of the population exist near or below subsistence level and the simplest types of technological improvement could, if properly applied, ease the lot of hundreds of millions of human beings. Modern science may not reveal the truth about the Universe or solve our ethical problems, but so long as people want H-bombs, space exploration, computer technology, and so on, or simply fast transport and more plentiful forms of energy and food; then our science will be needed because it alone can deliver the goods. A philosophy of pragmatism still has a lot to be said for it, but it is not much discussed here, except as a rather unimpressive spin-off of what the author dismisses as "soft-core" scientism — a less noxious type of scientism in comparison with the "hard-core" variety which maintains that science (especially biology) provides a morality (a "moral norm") of its own based on evolution and the "natural behaviour" of animals.

This is a stimulating and thought-provoking book which is worth reading in depth by all scientists who want to consider seriously the meaning of what they are doing and who wonder why they are sometimes pressed to discuss the philosophical implications of their work and not restrict themselves to its immediate practical results or just fiddle around with an intellectually amusing toy. Our science would be in a better position to defend itself if there were more scientists who can react to Professor Stent's arguments and at least understand what it is *not;* and who can realise, with A.N. Whitehead, that despite (or along with) all its practical achievements and intellectual magnificence, we are liable "to forget how strained and paradoxical is the view of nature which it [modern science] imposes on our thoughts". □

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Exercises in the traditional style

John Ziman

Teleology Revisited. By Ernest Nagel. Pp. 352. (Columbia University Press: New York and Guildford, UK, 1979.) $25.

THE official theology of science — as it might be enacted for example by the Council of the Royal Society if they were so wrongheaded as to ignore their own statutes forbidding such exercises — is empirical rationalism. For several centuries philosophers have been trying to elucidate and validate this creed. Of course, like any successful cultural movement, science really justifies itself by its works. But there is a species of intellectual who is not satisfied with the appearances of power, and tries to draw back the curtain hiding the machinery of science and show that it really does deserve our confidence.

In the past twenty years or so, these philosophers at the court of science have suffered a rude shock: all that they could find behind the fearful mask of the Wonderful Wizard was a little old man, gentle and wise but with no hot line to the Absolute. Critics such as Karl Popper, Norwood Russell Hanson and Thomas Kuhn have effectively demolished the claims of the scientific method as a means of arriving at certain truth.

For the older generation, this movement of Reformation has been an unhappy experience. Should they trendily embrace the latest doctrines, or should they stick with familiar beliefs that they cannot honestly discard? Professor Nagel retains "the outlook of an unreconstructed empirical rationalist", which he defends with characteristic wisdom and tolerant precision of thought and expression in this collection of essays. Although these were published independently over a period of more than 40 years, and are not artfully melded into a continuous narrative, they are remarkably consistent and coherent in style and content. If their outlook would be stigmatised as old-fashioned by the Younger Turks, it is not reactionary, and is very far from outmoded as a practical faith for the working scientist.

In fact, there are truths here that must be reckoned with. In the earlier chapters he brings to a halt the sloppier elements in Popper's fallibilist attacks on the traditional position. It may not be possible to define a general distinction between theory statements and observation statements, so that all science is in principle conjecture. But that does not mean that scientists are talking nonsense when they make such distinctions in the course of their research. Nagel acknowledges Popper's contributions in other respects, but repulses what he aptly calls the "Mephistophelian view of science . . . according to which to suppose even for a moment that any problem is really solved is to lose one's soul". And he annihilates Paul Feyerabend's irregulars after their wildly undisciplined charge to the cry "anything goes".

On the other flank, he repudiates false allies. The critique of Carnap's attempt to construct a logic of inductive reasoning is polite and detailed — but devastating. The mathematical theory of statistical inference has, I think, made some technical progress in recent years, but no intellectual structure can be safely founded on a symbol that is supposed to stand for the number of primitive predicates that might hypothetically be postulated concerning some body of evidence. To put it more succinctly than Nagel, it all seems to be a question of knowing how many beans make five. Empirical facts do not support theories by any device of strict logic.

Nevertheless, within these limits, his position is very open to attack from quite another quarter. That is to say, his genuine concern for the realities of the research process is applied solely to the arguments by which scientific knowledge is justified in the public literature. He admits that "assured knowledge in science is the product of a community of thinkers, each of whom is required by the tacit traditions of his group to be unsparing in his criticisms and evaluations of the cognitive claims presented to him" (page 13). But he does not seem to realise the corollary — that the scientific community may be (usually quite reasonably) persuaded to accept a cognitive claim on evidence that is much less watertight logically than philosophers would like. Nagel quite ignores the element of what Michael Polanyi called "tacit knowledge" in the assessment of research, and makes no reference to the social relations of the scientific community where this non-logical expertise is valued and shared.

In this light, the earliest essays in this book (dating from the 1930s) are of peculiar interest and significance. They show in detail, through the history of algebra and geometry, the pressure that can be exerted on an empirical science by the demand for logical consistency and completeness, The paradoxical appearance of imaginary numbers and imaginary points gradually drove nineteenth-century mathematicians into the modern stance, where each branch of pure mathematics is regarded simply as a system of relations derived conventionally from a prescribed set of axioms, without reference to any particular reality. In every stage of this process, extra power is gained by the increased generality of the results; but the outcome can only be correctly stated in Bertrand Russell's dictum that the pure mathematician does not know what he is talking about nor whether what he says is true.

Empirical rationalists have always been strong supporters of mathematical theory in science. They are very conscious of the value of reliably rigorous logical passages in scientific statements. But the more we purify a mathematical formalism, the more we come to realise that it is only the *medium* of communication between scientists and does not of itself constitute a *message* about the natural world. The empirical element in a scientific statement can only be expressed intelligibly in natural language, whose categories are inevitably somewhat vague. It is a pity that Nagel's remarks on this point were written before Stephan Körner had demonstrated its deadly significance for any strong logic of scientific justification.

What is also missing is a realisation of the dependence of scientific communication on non-logical human capabilities such as pattern recognition. It is true that a geometrical theorem can only be proved generally by the juxtaposition of abstract symbols for logical relations: but for most practical purposes the old Euclidean method, with its reference to diagrams, is suffiently convincing. From experimental high energy physics to sociobiology, every branch of the natural sciences is knit together by communications conveying diagrammatic inferences that cannot be interpreted by any known logical formalism but which are perfectly intelligible to another human being.

In the real world, it is the consensus that can eventually be reached by systematic intersubjective communications that defines our standards of objectivity and rationality. This applies at every level, from the everyday world of tables and chairs to the hidden world of quarks and neutrinos. It also applies to metascientific discourse on such questions as the relationship between theory and observation, or between cause and function. The point is that words like these are thematic rather than categorical. Like a picture, or a diagram, or a familiar model, they can convey very important ideas which cannot be defined, or paraphrased briefly, or precisely, or entirely unambiguously. They can only be understood in the light of experience, not of formal logic. In the final essay, Professor Nagel struggles to define the concept of teleological explication in biology, and effectively admits at the end that it was an impossible task.

Nevertheless, such strenuous exercises in the traditional style remain highly instructive for the research scientist, who must surely continue to believe, along with Professor Nagel, that "the logical methods of the modern natural sciences are the most effective instruments men have yet devised for acquiring reliable knowledge of the world and for distinguishing warranted

claims to such knowledge from those that are not". This (as the Council of the Royal Society would correctly perceive) is the faith upon which science is built, and the disciplinary code, at platoon, regimental, and divisional level, which binds it together in its war on ignorance and error. And yet there are perhaps occasions when such simple loyalties fail us, and the direction of the Army itself has to be called into question. □

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Updated science policy almanac

Austen Albu

Science and Government Report International Almanac, 1978-1979. Edited by D.S. Greenberg and A.D. Norman. Pp. 368. (Science and Government Report, Inc., P.O. Box 6226, 3736 Kanawha St NW, Washington DC, 20015, 1979.) $54.

It can be assumed that any study published today on government policy and organisation for science will be more concerned with technology than with pure science. Whether in captialist or communist countries this represents a victory for those pre-War marxist scientists who advocated planned support for science applied to utilitarian ends. The new edition of this valuable report, covering a period when economic difficulties restrained the expediture of most countries on R&D, makes it clear that pure science plays a decreasing role in government support policies compared with applied science and technology. Even Israel, which claims high support for basic research, based on its traditional approach to learning, has seen a significant increase in the proportion of applied research and development in the past few years. Although they do not all follow the same pattern, these national reports give an invaluable picture of recent developments in public policy and attitudes, and in changes in organisation for scientific advice and the carrying out of policy.

All governments support R&D, but the degree to which they do so financially and the organisations they use for the purpose vary a good deal. Britain and France seem to be among those countries with the most centralised structures among the advanced industrial nations. Even the USSR, if one forgets the overarching position of the Party, seems to have a wider range of institutions, although they are constantly changing. The growing emphasis on R&D to improve industrial production seems to have led to some decline in the prestige of the Academy of Sciences in face of the State Committee for Science and Technology.

In the United States the growth of anxiety in official circles about the country's apparent diminishing capacity for industrial innovation was one reason for President Carter's proposals to raise the rate of growth of funds for R&D, especially basic research. Congress, however, had rather different ideas and kept the 1979 expenditure to a total only fractionally more than that of 1978. One of the results of the increased involvement of governments in science and technology is the politicising of the decision-making process. In that most democratic of federations, Switzerland, the voters in referenda have displayed hostility to expansion of the universities and to subsidies to industry for R&D. This may be of little importance as Switzerland has the highest per capita rate of R&D expenditure in the world; 80% of it by private industry. Apart from wishing to curtail government expenditure, some voters in most countries are becoming increasingly interested in 'ethical' aspects of science or questions of the effects of technology on 'life style'. The main targets at present are nuclear energy and recombinant DNA. The former is an obvious embarassment for governments struggling with energy problems and no doubt adds to the growing part energy plays in national research programmes. Popular interest in the supposed dangers of recombinant DNA has been greatest in the USA and UK; in most other countries it has only attracted the attention of scientists and administrators.

The richer countries are concerned with problems of health research, both its changing nature and its accelerating cost. In the USA the Secretary of the Department of Health, Education and Welfare addressed scientists last year on the need to devise "health research principles" to be used for planning a "rolling" five year budget for the Department's programmes. The Federal Republic of Germany has published a programme of Promoting Research in the Service of Health, 1978-1981, of which an extremely interesting excerpt from the English translation is reproduced. It includes an examination of the difficulties of health research and a detailed list of fields in which greater R&D efforts would seem to be necessary.

So far the Trades Union in most countries have not shown much sustained interest in the problems involved in the results of scientific research. Only in the Scandinavian countries have permanent bodies been established by the Unions to keep them under survey. In France the scientific associations acted as pressure groups during the Presidential election of 1979 in which science policy played a prominent part.

Some rather unexpected figures in the output of scientist are revealed. In Sweden there was a fall in the output of Doctorates in the Natural Sciences between 1970 and 1976 from 461 to 172. In Britain, during the period of university growth in the 1960s scientific subjects got left behind, so that the "swing away from science" in the 1970's seems by contrast fairly modest. On the other hand engineering, at least in numbers, kept pace with university expansion. This, however, does not take account of a rapidly growing number of foreign students. The most unbalanced figures come from India which, in 1977, produced 140,600 graduates in the Natural Sciences and 15,000 in Engineering; bringing the total number of science graduates in the population to 1,182,272 of whom 16.1% were unemployed and of engineers to 251,860 of whom 2.4% were unemployed.

The most significant differences between nations lie in the ratio of government to private industry financed R&D and the proportion of military expediture. This may provide at least a partial explanation for the decline of British industrial performance. In Britain, France and the USA goverment provides more than half the nation's R&D funds; in Germany, Italy, the Netherlands, Sweden and Canada between 40 and 50%. In Japan the figure is about 30%, but Japan has the third largest R&D programme in the world. What is more significant is the fact that in the UK annd the USA roughly 50% of government R&D funds are spent on military programmes against a EEC average of 23%, a German figure of 12 and Japanese of 2. In all the major Western countries except the UK the share of government R&D spent on military programmes declined during the 1970s. In the UK it increased between 1970 and 1976 from 41 to 48%. If research expenditure is to be reduced these figures indicate where the possiblities of cuts should at least be scrutinised. On the other hand it seems that R&D activity by industry has been declining in the UK while that of the UK's main competitors has been rising; a matter which must be of concern to a government committed to reversing the decline of British industrial competitiveness.

This is the second year of publication of this important almanac. As an essential source of reference for scientific administrators, politicians and students of the subject it is to be hoped that it has now become firmly established in the rather overgrown forest of journals dealing with science policy. □

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New myths for old

Jon Beckwith

From Genesis to Genocide: The Meaning of Human Nature and the Power of Behavior Control. By S.L. Chorover. Pp. 238. (MIT Press: Cambridge, Massachusetts, and London, 1979.) £9.35; $14.

IN recent years a number of controversies have erupted over theories and practice in the biological and medical sciences. These debates include the subjects of genetics, race and IQ; the XYY male; sociobiology; psychosurgery; behavioural control practices in prisons; and the giving of drugs to school children said to be suffering from minimal brain dysfunction. A number of books have already appeared which deal with most of these issues from a critical perspective, including notably Kamin's *The Science and Politics of IQ* (Halsted: New York, 1974), Schrag and Divoky's *The Myth of the Hyperactive Child* (Dell: New York, 1975) and Ann Arbor Science for the People's *Biology as a Social Weapon* (Burgess: Minneapolis, 1977). However, nowhere, to my knowledge, has there been any extensive attempt to draw connections between all of these controversies. Now, Stephan Chorover, in this remarkably concise piece of writing (208 pages of text), for the first time, places all of these issues in a common framework and describes them in terms of a long historical tradition which preceded them.

Chorover, a psychologist at MIT, with a long-standing interest in abuses in psychology and psychiatry, unites what might seem to be disparate subjects by analysing them in terms of "meaning and power" in the realm of behaviour control. By these terms he refers on the one hand, to those mythologies, religious doctrines or scientific theories, which have served to structure people's view of their place in the world. ". . . what people believe about the essential nature of human beings has a powerful influence upon social expectations. Such beliefs tend to shape the ways in which people in a given social context are treated, and these in turn, significantly influence how they behave." Thus, ". . . ideas about human nature . . . deserve to be regarded as powerful instruments of behaviour control in their own right". "Power", on the other hand, refers to the power of certain groups in a society to apply these theories in practical ways for social control. The extreme example is, of course, genocide practiced by the Nazis based on biological theories of racial inferiority and superiority.

The book abounds with other historical and contemporary examples. Perhaps two of these will give some feeling for the analysis. In 1851, Dr Samuel Cartwright, a respected Louisiana physician, published a report commissioned by the Louisiana Medical Association purporting to explain among other things why there were runaway slaves. The roots of this problem, according to the doctor, lay in a disease of the mind which he termed "drapetomania", "the insane desire to wander away from home". Various punishments of the runaway slave could then be considered merely as medical treatments.

In a contemporary case study, Chorover describes in detail the story of "Thomas R." a portion of whose brain was removed during the heyday of psychosurgery in the late 1960s. Among other frightening stories associated with this case, he reports that the surgeons concluded that Thomas R. was psychotic partly on the basis that he was convinced that his wife was "carrying on with a neighbour". However, after the surgery took place his wife filed for divorce and eventually married that same neighbour. Thomas R., himself, has been socially non-functional since the operation and has spent much of his time in mental institutions.

Chorover, in reviewing the long history of what are essentially biological determinist theories, goes back to what he refers to as "Plato's lie". This term refers to the theory presented in *The Republic* that different people are born with different qualities of metals (gold, silver or brass and iron) mingled into their bodies, and that the metals in turn determine what status in society individuals will occupy. According to Plato (through Socrates) such a lie is necessary to justify existing social stratification. To Chorover "the basic texture of human nature mythology remains everywhere and always the same and . . . Plato's myth of the metals may be considered a prototype for todays myths".

It must be asked, of course, whether modern science has provided a much more solid basis for theories of human nature than earlier religions and mythologies. Chorover's answer is a clear no. He points to arguments used in the nineteenth and early twentieth centuries in the name of science (eugenics, phrenology, craniology, and so on) which we now recognise as totally without foundation. As a curious means of self-justification in the face of their critics, practitioners of these sciences have always likened themselves to Galileo, persecuted for trying to present the truth. Of course, Galileo, as Chorover points out, was attacked because his theories challenged the established order of things, whereas the makers of what Chorover generally calls sociobiological theories have, for the most part, provided rationales for existing social arrangements. He sees contemporary sociobiological theories as having no more solid scientific

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basis then their predecessors and laments the "inability or unwillingness of scientists to examine the social preconceptions inherent in the organization and conduct of their own work."

Although Chorover has done an impressive job of drawing the connections between his different topics, there are times when the attempt to unite all of the issues seems strained, as represented, for instance, by the title of one chapter "Genocide: The Apotheosis of Behavior Control". Further, although the role of sociobiological theories in justifying an inferior status for women is mentioned at several points, this subject deserves much more extensive treatment, particularly in the light of the recent resurgence of such ideas. These defects, however, do not seriously detract from the unusual service that this well-documented and well-referenced book performs for biologists, social scientists, psychologists and the public at large. For, to this reader, one is not equipped to evaluate these issues as they appear without an analysis which considers the social context and historical background to particular questions at the interface of science and society. □

Jon Beckwith is Professor of Microbiology and Molecular Genetics at Harvard Medical School, Boston, Massachusetts.

Instantaneous expertise

Thomas H. Jukes

Malignant Neglect. By the Environmental Defense Fund and Robert H. Boyle. Pp275. (Alfred A. Knopf: New York, 1979.) $10.

THE dust-jacket of this book modestly states that it "is based upon the enormous resources and scientific knowledge and authority of the Environmental Defense Fund (EDF), the nation's leading force in the field of environmental health". One author is a journalist, one a biochemist, one a science associate, and the qualifications of the other four are not listed.

A normal metabolite, acetaldehyde, is called a "known or suspected carcinogen" (page 105), and ammonia a "toxic organic vapor". The familiar litany is recited that no level of a carinogen is insignificant, and that the latent period for development of cancer is 5-40 years (page 6). Actually, the risk and length of time required for appearance of symptoms are both dose-related.

A graph (page 21) compares increase in cancer mortality rate for 1940-1975 (uncorrected for smoking, page 214) with increases in production of plastics and other organic chemicals. Compared with plastics, similar increases have occurred in skateboards, tomato production and pocket calculators.

Chapter 3 is about polychlorobiphenyls (PCBs). There is no lack of public information on this topic. But what is the record of the EDF on PCBs? Jensen's publication in 1966 (page 64) attracted wide attention to contamination by PCBs. The EDF virtually ignored PCBs and campaigned against DDT, so that in 1969, the "end of the ocean" by 1979 was predicted as a result of the arrest of photosynthesis in marine algae by DDT. However, Harvey *et al.* in 1973 reported that PCBs were present in surface water of the North Atlantic Ocean at about 20 p.p.10^{12}, and the level of DDT was less than 1 p.p.10^{12} Scott found in 1975 that DDT, 100 p.p.m. of diet, had no effect on hatchability and shell thickness in laying hens, as compared with lethal effects produced by PCBs at 20 p.p.m. PCBs were dumped promiscuously, because they were never subjected to the justifiably careful regulations that govern insecticides.

Page 102: "Driving through New Jersey... On U.S. 1, ... one begins to detect a pungent odor, like that of rotting friut. At first the odor is faint, but as the car swings into the New Jersey Turnpike, the smell grows stronger... Closing the windows does no good; the stench pours through the vents. The sky become visibly darker, like the twilight or an eclipse of the sun. At last, ... the source comes into view. It is perhaps the largest complex of oil refineries and petrochemical plants in existence... At night the flares atop the refineries produce an eerie light that transforms the scene into something from Dante's *Inferno*... Fourteen thousand poeple die each year from cancer in New Jersey".

On September 24, 1979, I drove south from Newark on the New Jersey Turnpike. I passed through the "refinery complex" at 6.45 p.m. There was no odour, there were no flares, and the only visible emission from the refinery was a few wisps of steam. To the west, there was a brilliantly clear sunset in which a range of hills, 30 miles away, was sharply etched against the sky.

Alarming statements are made about diethylstilboestrol (DES) used in meat production, and it is said (pages 149-151 and 219) to be detected in 1-2% of table meat and in pancreas. It has been detected only in liver, and only 0.6% of 8,293 beef livers sampled contained more than 0.5 p.p.b. The DES intake from meat is less than 0.001% of the equivalent daily oestrogen production by a nubile woman. Page 151 infers erroneoulsy that nitrofurans are antibiotics, and states "Even minute residues of antibiotics in meat and poultry can sensitize people and cause allergic reactions, such as rashes and shock, when one of these antibiotics is medically administered". I know of no evidence for this. Insufficient emphasis is given to the carcinogenic role of alcohol.

Arsenic and chromium, listed as

carcinogens, are nutritionally essential trace minerals, which is not mentioned. Either there is a threshold below which such substances are not carcinogenic, or there is no threshold, and therefore life is carcinogenic, but we cannot deny *both* of these possibilities. But the authors repeatedly emphasise that there is no safe dose of any carcinogen, and, on page 211, they say "'Life' is not carcinogenic". I disagree. First, there must be a threshold for certain carcinogenic stimuli (such as iodine deficiency in rats). Second, life can be carcinoginic because mutations accumulate in DNA during ageing, including mistakes in DNA replication and those caused by environmental mutagens.

Appendix A says that caffeine "may cause miscarriages or birth defects". Such a statement is pointless without being quantitative and is also irresponsible and sensational. The final chapter is "The Solution to the Problem", emphasising pages 224-232) "Personal Involvement in the Regulatory Process".

Leading experts in the field of environmental cancer are far less dogmatic than are the EDF sextet. John Higginson concludes (*Science*, **205**, 1363; 1979), that the manner in which people live — diets, dress, sexual and child bearing patterns, and especially tobacco and alcohol consumption — account for the great variations in cancer rates in different countries, and may be far more important than chemical pollutants. He reports that cancer patterns within Europe show no correlations with industrialisation. He says... "people would love to be able to prove that cancer is due to pollution or the general environment. It would be so easy to be able to say let us regulate everything to zero exposure and we have no more cancer. The concept is so beautiful that it will overwhelm a mass of facts to the contrary". □

Thomas H. Jukes is a Professor in Residence in the Department of Biophysics and Medical Physics, and a Lecturer in Nutritian Sciences at the University of California at Berkeley.

Potency of interferon

D. C. Burke

The Interferon System. By W. E. Stewart. Pp.421. (Springer: Wien and New York, 1979.) DM98; $53.90.

THIS book is a comprehensive review of the entire interferon literature, and fills a considerable gap. The last single-author book on interferon appeared in 1969 and the last multi-author book in 1973. Since then a number of specialist reviews have appeared, but no-one until now has had the courage and energy to tackle a survey of the field single-handed. The author is to be congratulated on a fine achievement, and the book will be greatly used.

As the author points out, interferon has received considerable recent publicity in both the scientific and popular literature as a potentially useful antiviral and antitumour agent. Its potential as an antiviral agent has indeed been obvious ever since its discovery by Isaacs and Lindenmann in 1957, but it has been slow to realise it. In retrospect, this is partly because quite inadequate amounts of interferon were available for the early trials, and partly because we did not choose the most suitable infections for treatment.

Attention has now swung away from treatment of respiratory infections to such life threatening conditions as chronic active hepatitis and viral infections of immunosuppressed or immunodeficient patients. Here useful and encouraging trials are taking place. It is, however, the potential use of interferon as an anti-tumour agent that has attracted most recent attention, for, stimulated by a careful ongoing trial against osteogenic sarcoma in Sweden, there have been several well-publicised reports of its activity against human cancer in the USA. In addition, interferon has emerged as a cellular modulatory factor, affecting the growth of cells, the immune system and the growth of tumours; and the mechanism by which it is produced is being investigated as a model for gene derepression in eukaryotes.

The book is therefore timely. Interferon research has continued apace and we greatly need a good critical summary. The book will be invaluable for those who are starting research in the area, but also for those who, like the reviewer, have been in the field a long time but who cannot remember who did what and when. The book has a complete coverage discussing topics as disparate as the structure of double-stranded RNAs, the genetics of the interferon system, the pharmacokinetics of interferon, and its role in normal recovery from virus infection, in addition to the trials as an antiumour and antiviral agent. The bibliography contains nearly 2500 references and the author has compiled 18 tables, many of which summarise information which has not previously been drawn together — for example, a compilation of the properties of type II interferon and a seven-page table of the cross-species activities of interferons.

However, the book is more than a compilation, for the author does not hesitate to comment, often constructively. There are, for example, interesting discussions of the different hypotheses which have been advanced to explain the mechanism of virus induction of interferon, of the mechanism of superinduction, and of the various schemes for interferon purification.

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Unhappily, in spite of the wide usefulness the book will undoubtedly have, the book is flawed by personal animus against a few scientists, and by occasional lapses into slangy diction. Cells do not "quit" making interferon, and sentences like "Studies on the mechanisms controlling the transcription and translation of interferons will likely soon enable better artificial regulation of these mechanisms, to provide sufficient interferon messenger RNA to allow its retrocopy into prokaryotic cells from which clones interferon can become truly abundant" make one gasp. □

D. C. Burke is Professor of Biological Sciences at the University of Warwick, Coventry, UK. He has worked on interferon since April, 1957, starting to collaborate with the late Dr Alick Isaacs and Professor Jean Lindenmann a month or two after the initial discovery.

Elements of cloning

Marie A. DiBerardino

Cloning: A Biologist Reports. By R.G. McKinnell. Pp. 130. (University of Minnesota Press: Minneapolis, 1979.) $8.95.

PROFESSOR MCKINNELL terminates his book with the following three sentences in the epilogue: "Cloning is one of many experimental procedures that provide information that may help in solving problems such as cancer and ageing. That is the why of cloning. And to assist people in understanding that "why — i.e., the rationale of cloning — I have written this book". The author has accomplished his objective. He is a research biologist who has carried out nuclear transplantation studies (one form of cloning) directed towards understanding normal cell differentiation, cancer, immunobiology and ageing. Few scientists possess both the authoritative background and literary talent to communicate accurately the relevance of bench-work research to its future potential for solving biomedical problems. McKinnell, however, manages to convey the significance of this basic research to the non-scientist.

McKinnell begins his book by correcting erroneous and inflamnatory statements about cloning and points out that the cloning of plants has been in practice for over 4000 years and has served to enrich man with beauty and food. This process is achieved by means of asexual reproduction resulting in a genetic replicate of the parent, whereas sexual reproduction involves the combination of genes from two parents and thus produces an individual containing a diversity of genetic traits.

The author then embarks on a description of classical animal studies carried out during the late nineteenth and early twentieth centuries that laid the ground-work for the amphibian cloning experiments. During this period there occurred a fusion of the disciplines of genetics, embryology and cytology; and the central problem that occupied these early scientists was the theory of nuclear equivalence. They sought to determine whether the diverse specialised cells of an organism retain the same set of genes as the nucleus of the fertilised egg. The earliest scientists recognised the stability of the differentiated state, that is, liver remains liver even if a person lives beyond ninety years, as pointed out by McKinnell. To explain this phenomenon, a theory of genetic differences among the progeny of the zygote nuleus was proposed by Weismann to account for the origin of cell diversity and stability. However, this proposal was refuted at least for nuclei from the earliest stages of development by the primitive cloning experiments of Loeb in the sea urchin and later of Spemann in the salamander. Finally, in 1952 "a fantastical experiment" termed and proposed by Spemann, was accomplished by Briggs and King. They sought to extend the primitive cloning experiments and transplanted a living nucleus from a cell from a frog blastula embryo, a day old, into a frog egg whose own nucleus had been removed surgically. The blastula embryo contained thousands of cells all derived from a zygote nucleus, but these blastula nuclei were still capable of promoting eggs to develop into normal larvae and eventually in later studies into normal frogs, demonstrating that the donor nuclei contained a set of genes equivalent to the zygote nucleus. This prototype experiment has been repeated many times in various amphibian species throughout the world, and it is now well established that many nuclei from early embryonic stages are *totipotent*. The theory of nuclear equivalence is still the central problem of developmental genetics today. The cloning studies have been most extensively carried out in two amphibian forms — the leopard frog, *Rana pipiens*, and the South African frog, *Xenopus laevis*. Cloning experiments have shown that during the course of embryogenesis, transplanted nuclei become progressively restricted in their ability to promote normal development of the eggs; and so far no nucleus of an *adult* cell has programmed an egg to develop normally. Despite this, a most extraordinary result does occur when nuclei from adult cells are cloned. A small percentage of eggs develops through embryogenesis to early larval stages and contain all the cell types and tissues of a larva, indicating that an extensive gene complement is still present and functional. Similarly, frog nuclei from adult kidney tumours when transplanted to eggs also lead to the formation of larvae. This subject, an area in which the author has made important research contributions, is discussed at some length. He points out that the formerly held pessimistic view of the irreversibility of cancer has been alleviated by these and other experiments. Thus, although important strides have been made in studying problems of normal cell differentiation and cancer, much more needs to be done in the future. McKinnell proposes: "If more were known about cancer-cell genetic material and about regulating its differentiated state, there would be the rational hope that a cancer chemotherapeutic agent could be devised that would regulate differentiation of these malignant cells".

Although amphibian cloning has been the most extensively studied among multicellular organisms, there has also been some work done on insects and fish. However, attempts at mammalian cloning have so far not been successful. But McKinnell points out that many of the techniques are available and the expectation that the remaining ones will be solved is encouraging. Cloning of farm animals may have agricultural advantages — for example, one could select for outstanding milk production, quality of wool, disease resistance, and so on; and this approach would be extremely faster than selective breeding for genetic traits.

Finally, McKinnell reviews some of the concepts of human cloning theorised by science-fiction writers, philosophers, theologians and lawyers. He addresses himself as a biologist and expert cloner to the social, ethical and biological problems inherent in their suggestions. Among his various refutations he emphasises that cloning may result in genetic replicates but not carbon copies, a widely misunderstood point. He also points out that species are successful due to the diversity of genes available through sexual reproduction, and so cloning is not a sound reproductive method.

McKinnell has performed an important service for both scientists and non-scientists in his well balanced and critical review of cloning, and at an appropriate time when both groups need an accurate account of the applications of nuclear transplantation studies to the biomedical sciences, in order to evaluate the erroneous and exaggerated notions of cloning that have been perpetrated upon the public. This book will be especially invaluable to non-specialists. A series of superb illustrations, together with the clarity and simplicity of prose, will permit the non-specialist to understand the basic elements of cloning and its biomedical implications.

Marie A. DiBerardino is Professor of Anatomy at the Medical College of Pennsylvania, Philadelphia. Dr DiBerardino began her nuclear transplantation studies with the originators of cloning (Briggs and King) during the 1950s.

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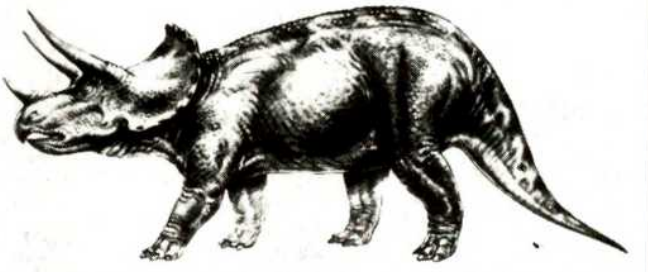
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Cytogenetics stages its own revival

Susumu Ohno

Human and Mammalian Cytogenetics: An Historical Perspective. By T.C. Hsu. Pp. 186. (Springer: New York, Heidelberg and Berlin, 1979.) Paperback $13.20; DM24.

IN 1959, Jerome Lejeune of Paris discovered the trisomy of chromosome 21 in those unfortunate individuals afflicted with the congenital syndrome then known as mongolian idiocy, now called Down's. With simultaneous discoveries by several British groups of the X0 sex chromosome constitution as the cause of Turner's syndrome in sterile women as well as of the XXY constitution in sterile men with Klineferter's syndrome, 1959 heralded the birth of human cytogenetics. Although the scale was obviously at least an order of magnitude smaller than that of the discovery of the DNA double helix in 1953, these discoveries, nonetheless, generated immense interest. In the heady day of an emerging molecular biology that was sure to become the core of the new biology, who would have thought that the venerable chromosome cytology of a distinctively pre-War vintage would stage its own revival? Furthermore, in so delicate a creature as *Homo sapiens*, who would have thought so gross a genetic imbalance as the trisomy of a whole chromosome could give rise to live births?

In spite of its belated birth, at least by 10 years in my reckoning, the revived old soldier did not immediately fade away. On the contrary, subsequent events made a long success story out of human and attendant mammalian cytogenetics. Soon, Lyonisation (inactivation) of one of the female's two X-chromosomes was established as the dosage compensation mechanism unique to mammals, and the discovery of evolutionary conservation, as opposed to innovation, of mammalian linkage groups in general, and the X-linkage group in particular, dramatically altered our view of the nature of natural selection. Somatic genetics based on cell fusion to produce interspecific somatic cell hybrids became the precocious child of human and mammalian cytogenetics. The technique of *in situ* hybridisation proved useful in localising prepeated DNA sequences on individual chromosomes. When an apparent senescence was about to start, Caspersson came to the rescue with the innovation of a chromosome banding technique.

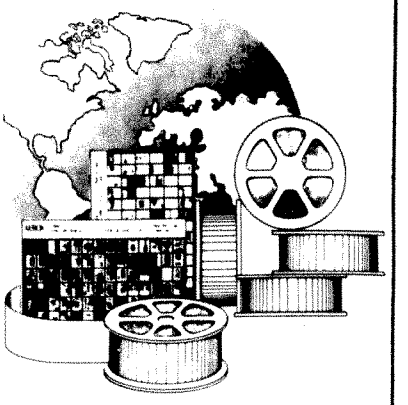
Now that senior members of the group involved in these initial excitements have passed or are approaching retirement age, one might expect a series of memoirs of those events. Professor T.C. Hsu of Houston opens up with a noteworthy salvo giving his own breezy and gossippy account. Of course, Rudolph Virchow's dictum *Omne cellule a cellule* applies equally well to scientific discoveries. The well publicised discoveries of 1959 were preceded by earlier ones; for example, the development of tissue culture techniques, the use of hypotonic pretreatments (T.C.'s own discovery) and colchicine, and the correct description of the normal human karyotype by J.H. Tijo and A. Levan that rectified earlier misunderstandings. Earlier still, a few outstanding investigators such as Sajiro Makino of Sapporo and Robert Matthey of Lausanne had accomplished a great deal in mammalian cytogentics without any of the above noted modern conveniences. In T.C.'s portrayal of those participants of the drama, one finds a number of witty gems; for example his accounts of Sajiro Makino and Kurt Benirschke.

It should be pointed out, however, that three participants of the same event are likely to give three entirely different versions of it. The fact is that the excitement of 1959 was essentially a European affair to which those of us across the Atlantic played second fiddle. Therefore, I await with great anticipation equally witty, but entirely different versions from some senior members of the European group.

Who should read T.C's memoir? I should say everybody, particularly those researchers of the younger generation — as recommended by M.J.D. White in his foreword, but for a different reason. I see one lamentable defect in the otherwise bright young generation brought up with the television screen. Because of their apparent abhorrence of printed words, the papers that they never read are unashamedly quoted and misunderstood and the writing style of scientific manuscripts is becoming entirely monotonous, as though they had been turned out by the same old computer. Julius Ceasar wrote his *Commentaries* and Augustus his *res gestae*. While nobody seems to care about them, almost everybody seems to know Claudius' gossippy memoir *à la* Robert Graves. If books like T.C.'s can rekindle the joy of reading in the heart of younger scientists, Professor Hsu should feel very proud. □

Susumu Ohno is Chairman of the Department of Biology at the City of Hope National Medical Center, Duarte, California.

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Core of controversy over biohazards

Sheldon Krimsky

The Recombinant DNA Debate. Edited by D.A. Jackson and S.P. Stitch. Pp.385. (Prentice-Hall International: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1979.) £14.55.

THE controversy of the past eight years over the regulation of recombinant DNA techniques has left in its wake some unusual and vexing policy issues. For the non-molecular biologist peering through the key-hole at the disputants, listening to a genetic vernacular that could pass as extra-terrestrial noise, the debate gives the appearance of a family feud. And it might have remained just that way were it not for the scientists who alerted the public to the potential hazards of their own work. Given the public's concern about this area of research and development, scientists may view similar acts of social responsibility as a danger to their disciplinary autonomy.

The principal target population affected by controls and regulation is a group of scientists engaged in basic research. Consequently, predominant expertise resides with a major interest group. The experts who are called upon to guide us through a safe technology path and on whom we must depend for objectivity can hardly qualify as impartial. Paradoxically, without them there could be no risk assessment.

The recombinant DNA episode holds many riches for science-watchers. It has stimulated discussion on a wide range of problems from public participation in the formation of science policy to the future prospects for human genetic engineering. In their edited volume *The Recombinant DNA Debate* David A. Jackson and Stephen P. Stich provide a series of essays that reach the central core of the scientific controversy over biohazards and, in addition, examine some of the moral and legal issues involved in the regulation of scientific inquiry. Stich, a philosopher, was on the faculty of the University of Michigan during the period the volume was in preparation. David Jackson, a University of Michigan microbiologist, was senior author of the first published paper on recombinant DNA techniques. Jackson also helped establish Genex, a US firm set up to develop commercial uses for recombinant DNA techniques.

The book, consisting of seventeen papers written between 1975 and 1977, is divided into three sections: Part I introduces the non-specialist to the techniques of genetic engineering; Part II examines the risks and benefits from various sides of the controversy; and Part III considers legal, social and philosophical issues. Most of the scientific contributors have also been principal actors in the debate (A.M. Chakrabarty, Roy Curtiss III, Bernard D. Davis, Joshua Lederberg, Robert Sinsheimer and George Wald). For this review I shall limit my comments to selected essays.

A.M. Chakrabarty reviews the areas in which recombinant DNA techniques may prove fruitful. These include drug, chemical and fuel manufacturing, crop improvement, and the treatment of genetic disorders. It is too early to judge whether or not Chakrabarty's optimism that recombinant DNA, by revolutionising the manufacture of biologically active proteins, "would make them available in large quantities at modest cost and would contribute significantly to the betterment of human health and welfare.". It is perhaps a symptom of the snail's pace of technology assessment that neither in this volume nor in the vast literature on this subject does their exist an analagous technical discussion of areas of potential misapplication or willful misuse of recombinant DNA technology.

Roy Curtiss III describes his construction of a strain of *E.coli* with twenty-four specific mutations designed to render it safe for molecular cloning. While initially an integral part of a strategy for biological containment, the skillfully engineered strain of *E.coli* (Chi 1776) may soon be irrelevant for the vast majority of experiments in the US. Recently, the Recombinant DNA Advisory Committee of the National Institutes of Health (NIH) voted to exempt most experiments from the NIH guidelines when ordinary laboratory strains of *E.coli* K12 are used. Little incentive remains for scientists to continue using the highly sensitive Curtiss strain despite its widely acclaimed safeguards.

Papers outlining the potential dangers of gene-splicing are contributed by Robert Sinsheimer, George Wald, and the recombinant DNA study group of Science for the People. Both Sinsheimer and Wald implore their colleagues to consider the long-term ecological consequences of this new-found power to rearrange genetic substance across widely divergent species. Their emphasis is on the possibility of unexpected events or "evolutionary booby-traps", in contrast to specific scenarios of danger.

As a counterpart to the DNA critics, there are contributions by Bernard D. Davis and Rolf Freter focusing on imagined, as opposed to real, dangers of the research. Davis invokes Darwinian evolutionary principles to argue that Nature abhors new pathogens. In his view, biological competition and natural selection operate in tandem to inhibit the establishment of novel pathogens in the ecosystem. Freter reviews the experimental data relevant to the question of whether *E.coli* K12 can be transformed into an epidemic pathogen. The evidence, he concludes, suggests that we cannot produce an *E.coli* superpathogen by recombinant DNA techniques. Nevertheless, he emphasises the importance of weakened hosts in gene-splicing experiments for the safety of laboratory personnel and recommends the use of *E.coli* strains incapable of growing in germ-free mice (a property of Chi 1776).

Two philosophical papers appear in Section III. Stephen Stich examines several types of moral reasoning voiced during the most active period of the debate. He challenges the view that scientific research should be curtailed if it can lead to the discovery of knowledge that might be disastrously misused. He believes that such a principle, if consistently applied, would paralyse all research: "It is hard to think of an area of scientific research that *could* not lead to the discovery of potentially dangerous knowledge." While Stich gives serious attention to the use of probability estimates of biohazards, he neglects to apply similar reasoning to the application of research programmes. The potential for misuse of science is not distributed uniformly among all research enterprises; consequently, some may warrant greater social control than others.

Carl Cohen, in his essay "On the Dangers of Inquiry and the Burden of Proof", wields a philosophical scalpel to the critics of recombinant DNA research. He skillfully dissects their arguments, exposing lack of rigour and their weak analogical reasoning and use of criticism, to which no response is possible ("heavy question arguments"). Cohen's reconnaissance of the terrain of criticism is limited to the most radical critics. Moreover he says nothing about the faulty reasoning and emotional responses of proponents of laissez-faire science. There, too, one finds arguments built on conjectures and quasi-scientific findings.

This is the first collection of papers on the recombinant DNA debate by a major publisher for a non-technical audience that addresses scientific, philosophical and policy issues. The essays are generally of high quality. They centre around the controversy in the US up to 1977. Since that time new issues have surfaced including the patenting of novel life forms, the rise of commercial interest in recombinant DNA techniques, the health of workers in large scale fermentation plants, and the disposal of by-products in production.

The weakness of this collection is in its failure to cover the political and sociological context of the debate. With the slight exception of an essay by sociologist Max Heirich on how the funding of science affects decision-making about science, there is no treatment of the key determinants that shaped DNA policy in the US. Factors of interest are: the

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effectiveness of the scientific lobby; the commercial ties of scientists in the recombinant DNA field; the control of the risk assessment process; the ability of scientists to contain the issues to laboratory biohazards; the role of environmental groups; and the politics of the Recombinant DNA Advisory Committee. We shall await another collection of papers to bring the issues up to date and address these critical factors. □

Sheldon Krimsky is Acting Director of the Program in Urban Social and Environmental Policy at Tufts Unversity, Medford, Massachusetts.

Synthetic biology

Richard Roblin

A Double Image of the Double Helix: The Recombinant DNA Debate. By Clifford Grobstein. Pp. 177. (Freeman: San Francisco and Reading, UK, 1979.) Hardback £6.90; $11; paperback £3.50; $5.95.

THE tone of books about the recombinant DNA policy debate has both reflected and fueled the intensity of public concern. As the debate grew heated during 1976–78, books by journalists and science-writers vividly detailed the early history of the controversy, and discussed possible biohazards. In the quieter atmosphere of 1979, Clifford Grobstein has contributed a balanced, analytical view of the American sector of the recombinant DNA landscape which emphasises the science and public policy issues thrown up by the turmoil. In doing so, he has written an excellent primer for the next stage of discussion about recombinant DNA policy issues.

Grobstein's book will be read with profit by a variety of readers. Here, interested members of the public will find an outline of the relevant science, a history of the controversy, and thought-provoking discussions of exploration, risk and research regulation. Scientists should find interesting Grobstein's interpretations of the social and political context of their research, even though they may not like the world which he describes. Bureaucrats and legislators should ponder Grobstein's comments on the incompleteness and instability of current public policy on recombinant DNA research. In Grobstein's opinion, failure to extend application of the NIH Recombinant DNA Research Guidelines to industrial as well as academic research risks undercutting their effectiveness.

One of the major sections of the book deals with the issues surrounding regulation of basic research. Grobstein finds such regulation generally distasteful, and articulates several requirements for sound regulation of research. He views continued research and expansion of human knowledge as "essential to the public interest", and would restrict these only when "there is compelling evidence of clear and present danger." However reassuringly familiar this criterion sounds to American ears, how would it apply in the recombinant DNA case? The conjectural nature of recombinant DNA biohazards remains the central intellectual dilemma of recombinant DNA policy. Except for intentionally malevolent applications, there never was, and may never be, compelling evidence of clear and present danger in the use of recombinant DNA techniques. What policy to follow given this uncertainty remains the central issue of the recombinant DNA debate. Does this mean that Grobstein believes the guidelines approach and the concommitant public and legislative furore to be an example of unsound regulation? He doesn't say, and I believe he would have made his book better if he had been more specific in this and several other places.

One thorny problem associated with the recombinant DNA debate is the role of the public in policy formation. If there are conjectured risks to public well-being because of the use of recombinant DNA techniques, then should the public have a say in how these techniques are to be used? Grobstein points out the tendency to apply criteria and procedures developed for regulation of human experimentation to recombinant DNA research. This analogy led some to suggest that appropriate policy for recombinant DNA research required something like a public 'informed consent' before proceeding with widespread use of the technique. Grobstein raises, but does not directly answer, the question of whether this extension of the principles of risk–benefit calculation and informed consent is appropriate for research in general (or recombinant DNA in particular). My own feelings are that these criteria are not appropriate where risks are conjectural, because meaningful risk–benefit estimations cannot be made. In addition, why single out the area of recombinant DNA research, when public "informed consent" has not been seriously urged in other research situations (for example, deepspace exploration) where risks could also be conjectured?

Inevitably, in an area as fast moving as recombinant DNA research, the pace of new discoveries outstrips the rate at which books can be conceived, written and published about them. Thus, Grobstein mentions only in a footnote the recently discovery that the DNA sequences which code for several eukaryotic viral and cellular proteins are not all adjacent to one another as they are in bacterial DNA. The discovery of 'split genes' and RNA splicing in higher eukaryotic cells, and the lack of evidence for RNA splicing mechanisms of comparable specificity in *E. coli*, have led many scientists to conclude that split eukaryotic viral and cellular genes will not be correctly translated into functional proteins in *E. coli*. If many eukaryotic genes are split and cannot produce functional proteins in *E. coli*, then the chance of producing pathogenic *E. coli* through 'shotgun' cloning of eukaryotic DNA segments is reduced still further. These arguments were partly responsible for the considerable reduction in containment for such cloning experiments in the December 1978 revision of the NIH Guidelines. It is unfortunate that Grobstein was unable to include the impact of this new information in his discussion of potential risks in recombinant DNA research.

Grobstein ends his book with a suggestion that a presidential study commission be appointed to work toward a comprehensive assessment of issues generated by the recombinant DNA debate. He feels that this would create a needed arena "in which all issues can be examined and which gives full access to all interested parties and experts". However, since Grobstein's book was written, there have been significant changes in both the content and administration of American recombinant DNA policy. Estimates of the possible risks of recombinant DNA experimentation have dropped further. Accordingly, containment requirements for several types of experiments have been reduced, and significant supervisory authority over recombinant DNA experiments has been delegated to local Institutional Biosafety Committees. The NIH Recombinant DNA Advisory Committee (RAC) has been enlarged to 25 members, thus expanding its expertise and diversifying it politically. Meetings of the RAC have become a national forum for debating and voting to recommend changes in the NIH Guidelines, the chief ingredient in current American recombinant DNA research policy. RAC procedures, which provide for prior publication of proposals to be discussed and publicly accessible meetings, have opened a permanent channel for public input into decision-making on recombinant DNA research policy. In addition, the major scientific and procedural objections of critics like Sinsheimer, Wald and King have either been accommodated or been found to be generally unpersuasive. In the light of these recent developments, one wonders whether Grobstein would continue to advocate a presidential study commission.

Given the current trend, we are at the beginning of a period in which the NIH Guidelines will be partially or completely

dismantled. Some scientists believe that it will be possible to stuff the genie of regulation of recombinant DNA research back into the bottle. If so, what kind of a legacy will the recombinant DNA debate leave? Perhaps it is too early to clearly assess this question, but the following thoughts come to mind. The development and evolution of guidelines for recombinant DNA research provides a case-study in innovative formation of a policy to cope with uncertainties generated by a major expansion in human knowledge and power. Early discussion of the possibilities in public, a continuation of cautious research coupled with risk-assessment studies, and built-in mechanisms for modifying regulations in the light of new knowledge may prove to be useful principles in analogous future situations. In addition, the recombinant DNA debate has conveyed to a wide audience the vast potential of the 'synthetic biology' opened up by recombinant DNA techniques. Thoughtful consideration of the ends to which this potential is being and could be applied should commence, as concern over potential biohazards diminishes. □

Richard Roblin was a member of the National Academy of Sciences Committee on Recombinant DNA Molecules and the organising committee for the 1975 Asilomar Conference on Recombinant DNA Molecules. He is now Head of the Molecular Biology of Tumor Cells Group, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland.

Drives and action patterns

John R. Krebs

The Origins and Rise of Ethology: The Science of the Natural Behaviour of Animals. By W. H. Thorpe. Pp. 174. (Heinemann Educational: London, UK; Praeger/Holt, Rhinehart and Winston/CBS: New York, 1979.) £5.90.

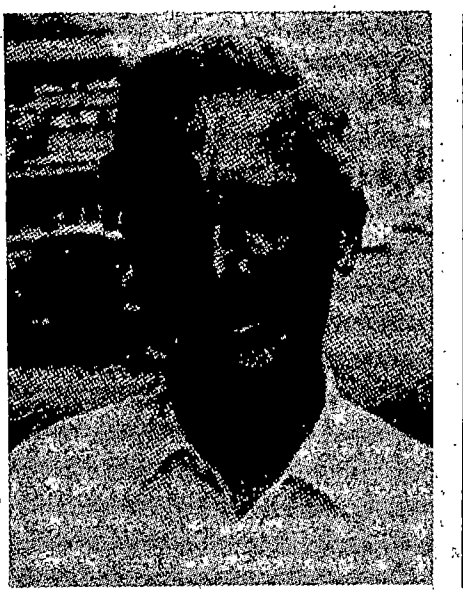
THIS book is an extended version of the first Niko Tinbergen lecture, given by Professor Thorpe to the Association for the Study of Animal Behaviour in 1974. He describes how ethology started, what it was like in the middle of the twentieth century and what it is about today. The middle section of the book, which is full of anecdotes and personal recollections of Professor Thorpe, is to my mind the best.

The early history of ethology is traced by Thorpe to John Ray, the seventeenth century British naturalist who, among many other things, did experiments on clutch size in birds. The subject in its present day sense (the naturalistic study of animal behaviour; not the "science of character-formation" as in the term used by J.S. Mill) emerged in France in the eighteenth and early nineteenth centuries through the writings of C.G. Leroy, J.B. Lamarck and E. Geoffroy-Saint-Hilaire. (Some readers may be amused to know that Leroy was gamekeeper of the area in France called Marly — virtually the same name as that given to the wood near Oxford in which R.A. Hinde carried out his thesis research nearly 200 years later.) Although Britain had a strong traditon of country naturalists in the eighteenth and nineteenth centuries, Thorpe rightly emphasises D. Spalding as the key British figure in the early development of ethology. Spalding pioneered experimental studies of behaviour development and had a great impact on Lloyd Morgan, who was influential in espousing a scientific, reductionist approach to the study of animal behaviour. Among early American students of behaviour, Thorpe mentions particularly the contributions of Whitman, Craig, Lashley and Wheeler; perhaps he should also have included W.C. Allee whose book *The Social Life of Animals* foreshadowed much that is current in ethological thinking.

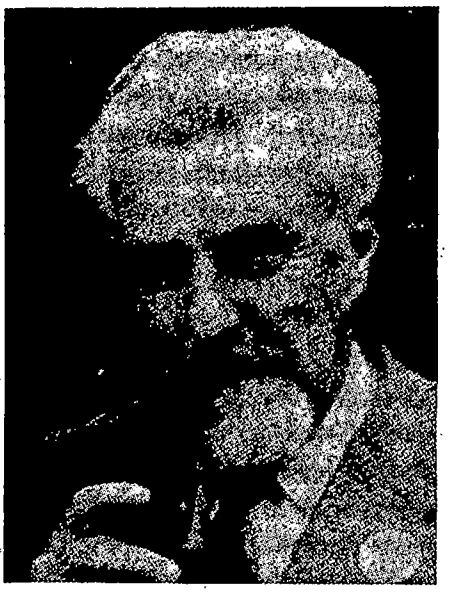
From this straightforward historical account, Thorpe moves to the period with which he is familiar from personal experience. In the 1930s and 1940s ethology became firmly established as a discipline distinct from other approaches to the study of behaviour, mainly through the work of Lorenz and Tinbergen. The account of this period is very revealing to those who have seen only only the later years of the era of these great men. Today, Lorenz's theories are viewed as somewhat old-fashioned and naive, but as Thorpe stresses "The very fact that Lorenz's early papers now appear so outdated is a tribute to their effectiveness . . . It is the impetus which they gave to experimental work which has, in a sense, rendered them so quickly out of date, although they are still of course extremely well worth reading".

According to Thorpe, the central problem facing ethologists in the 1930s was to elucidate the nature of instinctive behaviour. Although it was widely recognised that animals perform complicated "goal oriented" sequences of behaviour, such as nest-building and courting, without any particular training, there was no conceptual framework within which to analyse and dissect these instinctive behaviours. Lorenz provided the framework in a series of papers published in the decade 1931-41. Instincts were viewed as being made up of simple stereotyped, inborn movements (fixed action patterns) linked together by more flexible and modifiable goal oriented behaviour (appetitive behaviour). Both of these were thought to be driven by "specific drives" (physiological mechanisms). Usually the fixed action patterns were released by a specific stimulus (releaser) and they were consummatory in the sense of dissipating the specific drive for that appetitive behaviour and fixed action pattern sequence. Internal drive was considered to then gradually build up until a fixed action pattern could discharge it again. If no suitable stimulus appeared, the action might go off "in a vacuum". Professor Thorpe stresses that this theoretical framework was valuable bacause it immediately led people to investigate in detail the nature of instinctive behaviour. For example, physiological and endocrinological studies of drives, perceptual studies of releasers, and genetic studies of fixed action patterns were set up to test Lorenz's ideas.

While providing a lucid account of Lorenzian theory, Professor Thorpe also tells us many anecdotes from the immediate post-War years. Stories are told of how Lorenz nearly went to Cambridge

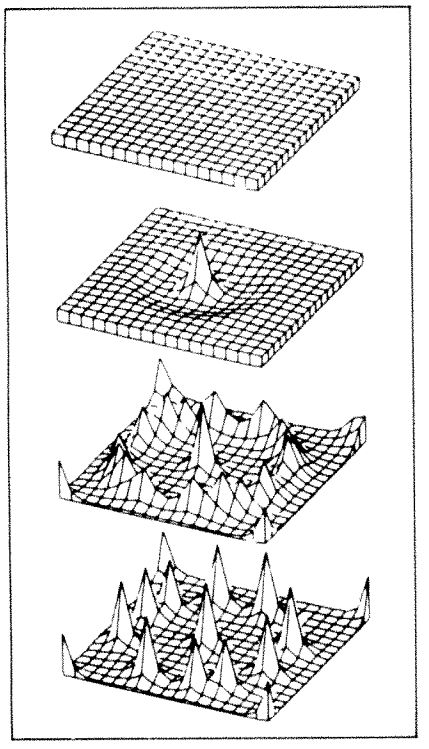


Niko Tinbergen and Konrad Lorenz in Cambridge, 1959



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instead of to the Max Planck Institute; how Lorenz and Tinbergen quacked like ducks to put off two predatory women; and how Thorpe himself was asked to present a paper of "about two hours" when he turned up at the First International Ethological Conference! It would be nice if this part of the book were expanded into a full volume at a later date.

The last chapter, on the present position of ethological concepts and research, is also fascinating from an historical viewpoint. It illustrates how the same events can be seen in a totally different light by observers from two different generations. In Thorpe's view, ethology today still works within the Lorenzian framework, and the questions asked still deal with fixed action patterns, drive, displacement activities, releasers, and so on. The details have changed very much, but the conceptual armoury is still the same. To an ethologist trained a generation later, the view is that ethology (in particular British and American ethology) underwent a purge in the 1950s and early 1960s, mainly due to the masterly critiques of R.A. Hinde; in this much of the earlier Lorenzian theorising was rejected and replaced with a hard-headed, reductionist approach. This created a spartan atmosphere in which theorising was definitely out, and hard data in. Then the early 1970s saw a flowering of new theoretical ideas and new questions about behaviour, this time linking ethology with population genetics and evolution. Perhaps there will be a new purge in the 1980s.

Neither of these contrasting views about present-day ethology can be said to be right or wrong: they emphasise that in spite of our attempts to be objective about the behaviour of animals, the interpretation of our own history is very much subjective. □

John R. Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

Selected genetics

M. J. Lawrence

Understanding Genetics. By E.B. Ford. Pp. 201. (Faber and Faber: London, 1979.) £7.25.

PROFESSOR FORD tells us in the preface that this book is an attempt to meet the needs of those who, whether they be men of general education or scholars in other fields of learning, wish to know something of the theoretical aspects of genetics. This is a laudable, though ambitious, objective, for if such an attempt is to succeed, the author will have to possess an unusually wide and deep knowledge of the subject, so as to be able to give both an adequate coverage of it and to deal with open questions in a fair and unbiased way. To what extent does Professor Ford's new book meet this requirement?

The book contains eight chapters which deal with the Principles of Genetics; Sex; Evolution; Polymorphism and Mimicry; Industrial Pollution; Genetics in the Countryside and Garden; Individual Variation in Man; and Genetics and Human Societies. The contents of the book reveal that Professor Ford's coverage of the subject is far from complete, for he deals only with some aspects of classical, human and what he has called ecological genetics. There is nothing about molecular or microbial genetics, despite the fact that these are now the most actively researched areas of the science and are, moreover, ones which raise problems of great interest to the layman. This omission, furthermore, is no mere oversight, because we are told that the structure of DNA is "much beyond what is appropriate for a book of this kind" (page 30). This is surely a serious error of judgement. The reader will look in vain, therefore, for enlightenment on why, for example, there has been much public debate on the subject of genetic engineering. For this they would have to look elsewhere.

Those who are familiar with Professor Ford's style will know what to expect from this book; nor will they be disappointed, for here again we find his characteristic, enthusiastic and authoritative treatment of the subjects with which he deals and also a number of amusing and illuminating anecdotes, including one about how to plan for a scientific camp. Indeed, the book seems to be derived from two of Professor Ford's previous books, *Ecological Genetics* and *Genetics for Medical Students*, and is therefore something of an intellectual biography of his work. Those who are familiar with this work, however, will also know that there is by no means general agreement on some of the matters that are discussed. For example, apart from the obvious exception of sickle-cell anaemia, there is almost no evidence to justify the confident statement on page 151 that "heterozygous advantage is the usual method for maintaining polymorphic variation", though it is, of course, possible that some polymorphisms are maintained in this way. Then again, we do not know "that it is usual for" selective advantages "to reach 20 or 30 per cent, often much more" (page 69). The layman, however, will be unaware of these biases and hence is likely to be seriously misled when he goes on, as Professor Ford hopes he will, to pursue his interest in genetics in other works.

There are, unfortunately, other errors of emphasis and, indeed, of fact in the book. For example, we are told on page 155 that "it would be highly *suspicious* [my italics] if a pair of blue- or grey-eyed parents had a child with dark-brown eyes". While such an outcome would be unusual, such cases have occurred in circumstances where the paternity of the child was beyond reasonable doubt. Then again, I know of no evidence to justify the statement on page 74, concerning the pairing of homologous chromosomes during the first division of meiosis that "allelic attraction must be stronger when the pairs are identical (homozygous) than when they are dissimilar (heterozygous)". Is it really the case that Man is more variable than other species (page 13) and that among our races, the Japanese are "much the less diversified" (page 172)? There is a particularly misleading error in the simple numerical example on page 28 which Professor Ford uses to explain how the order of genes along a chromosome may be determined from the results of a three-point backcross (his ACB should be BAC). On page 57 we are told that if "we keep organisms in a strictly constant condition in the laboratory, all their variation *is* [my italics] genetic" (would that this were true!). The simple method for the detection of heterozygous advantage given on page 107 is unsatisfactory because the χ^2 in question can be significant in the absence of this type of selection. Lastly, on page 116, where Professor Ford writes, about self-incompatibility in those species in which this is determined by a single, multi-allelic gene and control of the pollen reaction is gametophytic, that: "If both the male and female plants possess, for instance, *S2* and *S2* (one carried by the pollen, the other possessed by the stigma) fertilisation fails". This statement is highly misleading because it is true if and only if the comment in parenthesis is ignored (if the latter were correct the cross would be compatible). There are a number of other errors in the book, including some concerning page and author references; and the way in which some of the subject material is dealt with in the last two chapters of the book made this reader uneasy.

Reviewing this book has been a painful task, because I, like many of my colleagues in the field of ecological and population genetics, owe Professor Ford a great debt; he is one of the pioneers of the subject and has made many distinguished contributions to it. But in view of the stated aims of the book, we have a wider duty to those people for whom it is intended. With this in mind, the book cannot be recommended to the layman. □

M. J. Lawrence is a Senior Lecturer in the Department of Genetics at the University of Birmingham, UK.

Illuminating astronomy

Owen Gingerich

Man's View of the Universe: A Pictorial History. By G.E. Tauber. Pp. 352. (Crown: New York, 1979.) $19.95.

TAUBER'S elucidation of "evolving concepts of the universe from ancient times to today's space probes" belongs to the genre of history of astronomy in the service of popularisation. He thus joins the class whose predecessors range from Camille Flammarion to Patrick Moore and Fred Hoyle.

An historical approach is particularly felicitous for astronomy, with its long and nearly continuous development. It has the advantage of starting with the simplest observations and theories, which can introduce the novice to the sky as it still appears. The story then leads through the drama and pathos of Galileo, to the puzzling and curious genius of Newton, and finally in a modern climax to Einstein and relativity. Tauber exploits the anecdotal and the picturesque to leaven what is essentially a popular textbook, and in so doing produces a guide to modern astronomy far more readable than the typical elementary textbook. He is obviously most at home with relativity and with cosmology, and there his presentation is outstanding. But the subtitle, *A Pictorial History*, promises us more than a mere textbook, and alas from either the pictorial or historical viewpoint the book is a considerable disappointment.

There are, to be sure, hundreds of illustrations, which in effect form a separate and parallel account, unreferenced and almost completely unlinked with the running text. Some of them are fresh and attractive, such as the pair of portraits of Wilhelm IV of Hesse and his wife, the Foucault pendulum in the Pantheon, or Robert Goddard beside an early liquid rocket. However, many are commonplace and often poorly reproduced; a significant proportion of the illustrations are line diagrams, which emphasise the textbook aspects to the detriment of the historical qualities.

Consider, for instance, the section on Newton: the best of the illustrations is a fine manuscript drawing of Newton's telescope, and there are halftones of Newton, Woolsthorpe, a modern painting of Newton with his prism, plus muddy views of Huygens and conic sections. But these are overwhelmed by a score of diagrams representing everything from the graph of an inverse square law, a hopelessly wrong scheme of recombining coloured light with a second prism, a redrawing of Newton's famous (and incorrect) diagram on free fall from his letter to Hooke, precession, tides, and so on. The Newtonian section epitomises the pictorial problems of the entire work: squeezed and uninviting layouts, erratic quality of reproduction, and uninspired typography. It would be heartless to dwell further on these inadequacies, for which the publisher is probably more to blame than the author.

Several pictures are erroneously captioned or credited. The widely copied traveller poking his head through the vault of the heavens to see the celestial gearwork beyond is here reproduced as a sixteenth-century woodcut, although it is now definitely established as late nineteenth-century art nouveau, probably from the hand of Camille Flammarion. It is hard to believe that the photograph purporting to be the Magellanic Clouds on page 241 really shows those objects. On page 36 there is "an artist's view (date unknown) of Thales' universe", in the public domain according to the list of credits. In fact, the figure was specifically drawn for Carl Sagan's Time-Life book, *Planets* (1966). The drawing that decorates the title page has been adapted from the same source.

As a pictorial *history*, Tauber's production fares even worse. Factual errors abound. Contrary to his text, there is absolutely no evidence that Copernicus had to occupy himself with astrology (page 98), and in Kepler's music of the planets, the faster the planet, the higher the notes (page 124). Galileo's telescope did not contain three convex lenses (page 196), and Herschel never became Astronomer Royal (page 212).

Actually such flaws are rather minor, and although they detract from the book's reliability, they scarcely interfere with its pedagogical exposition. What dismays the historian of astronomy far more than a peppering of factual errors is the writing of "Whig history". That expression, introduced by Herbert Butterfield, refers to history seen from the vantage point of the victor; in the history of science, it means evaluating the past in the light of today's theory. It is Whig history of science, for example, to give the ancient heliocentric schemes of Aristarchus any special prominence. As physicist-historian Stephen Brush has said, credit for discovery should be given not so much for the originality of the concept as for the persuasiveness of the arguments; certainly



Galileo Before the Holy Office by J. N. Robert-Fleury, 1847

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the single sentence that has come down from antiquity about Aristarchus' cosmology contains no persuasive arguments.

Tauber gives a column to Aristarchus but his Whiggishness is generally more subtle, precisely the kind that distingushes textbooks and popular science from history. In describing the distances of stars, he goes straight to the trigonometrical parallaxes finally established by 1840, thus ignoring the intellectual breakthrough of the late seventeenth-century when astronomers recognised that stars were really distant suns and when they began to calculate reasonable distances photometrically. Similarly, in telling of Huygens' discovery of the rings of Saturn, he places this in the context of building better telescopes, not realising that the Dutch astronomer made his interpretation in mind's eyes at a time when the ring system was actually edge-on and invisible.

Tauber's presentation is rich in chronological facts rather than the historical facts that illuminate the actual process of discovery. In introducing galaxies under the heading "Island Universes" he alludes to Kant, Messier, the Herschels and Lord Rosse in a swift succession leading to the Shapley-Curtis debate in 1920.

He refers implicitly to the rotations of spiral nebulae found by van Maanan, but by Whiggishly suppressing explicit mention of these now-discredited measurements, he plants a confusing discontinuity into his story. Nor does he mention novae or red shifts, because he sorts these key elements in the historical account into other bins. There is nothing reprehensible about organising the material this way for instructional reasons — indeed, it probably leads more rapidly to an understanding of our modern ideas of the Universe.

In contrast, it is the task of the historian of science to show the development of science as a creative human activity, which for past science requires a sympathetic reconstruction of the motivations and limitations of a bygone period; this demands a selection of those facts that illuminate the understanding at that time, not the rather different group of facts now woven into our contemporary knowledge. I suspect that most prospective owners of Tauber's *Pictorial History* will be quite satisfied to get a relatively painless and accurate survey of modern astronomy, and would probably be disappointed if they got serious and accurate history of science. My plea to my scientific colleagues is that Tauber's work should be seen as a somewhat successful popularisation of science, but not as history of science. □

Owen Gingerich is Professor of Astronomy and of the History of Science at the Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts.

Prehistoric Avebury

R.J.C. Atkinson

Prehistoric Avebury. By Aubrey Burl. Pp. 275. (Yale University Press: New Haven, Connecticut, and London, 1979.) $19.95; £8.95.

NEXT to Stonehenge, Avebury is better known than any other prehistoric monument in Britain. About Stonehenge countless books have been written, good, indifferent and frankly lunatic; but about Avebury very little, even though, in the words of its first recorder, John Aubrey, it "does as much exceed in bigness the so renowned Stonehenge, as a Cathedral doeth a parish church". It has not escaped, even recently, the weird and uncritical lucubrations of the lunatic fringe; but until now there has been no comprehensive treatment of Avebury and its archaeological setting which could rival William Stukeley's *Abury* of 1743. This is not to ignore, of course, Dr Isobel Smith's *Windmill Hill and Avebury* (Oxford University Press, 1965) or *The Avebury Momuments* (HMSO, 1976) by the late Faith Vatcher and her husband, which are primary sources; but the first is an excavation report, and the second a concise guide for the visitor.

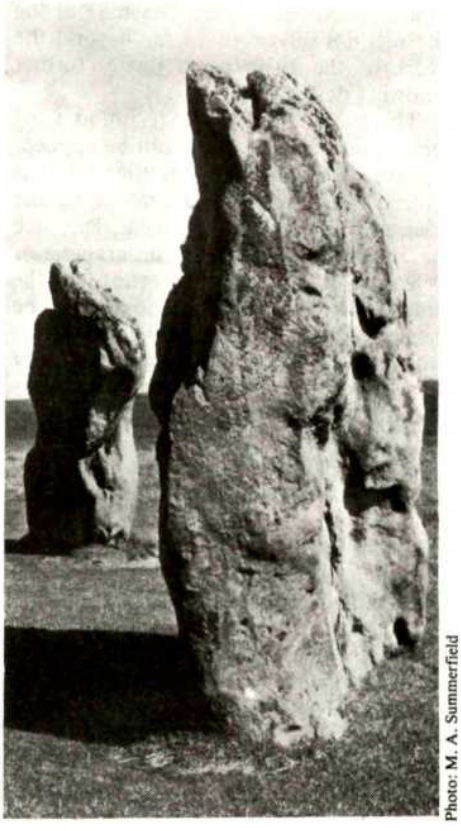
Aubrey Burl has now turned from British stone circles in general, on which he is the main authority, to a detailed study of one of the best known of them, on a scale and with a breadth of knowledge and vision rivalled only by Stukeley. This is a quite remarkable book, and a pleasure to read. It is intended for the intelligent layman, and in its presentation it has many of the attributes of more expensive 'coffee-table' books, with large format, well-spaced type and lavish illustrations, including more than a dozen full-page colour plates; but it also contains a very full set of notes and references, a comprehensive bibliography and a well-designed index.

The specialist reader (and maybe others as well) must make full use of the index, because the treatment is markedly discursive and the discussion of parts of Avebury, and of neighbouring sites, is widely dispersed throughout nine chapters. These are thematically arranged, with an underlying but not explicit chronological order, which is not that of the chronology of Avebury itself. As one familiar with the monuments from childhood, and with something of their archaeology from adolescence, I find this confusing and sometimes irritating; but I am not perhaps the reader Burl had in mind.

Throughout the book Burl has used his very wide reading to draw parallels between the available archaeological evidence, from Avebury and neighbouring sites, and sometimes from more distant sites in Britain, and ethnographical observations from other parts of the world, principally the documented customs for the living and the dead amongst various tribes of North and Central American Indians.

Some of these likenesses are so striking that the lay reader may be tempted to fall inadvertently into a logical trap, and to suppose that "because it could have been like that, therefore it was". Burl is obviously aware of this danger, and has taken some care, though perhaps not quite enough, to warn his readers of the pit-falls of facile conclusions based on comparisons of very different contexts in time and space, and of very diverse economies and environments. In the end ethnographic parallels can serve only to enlarge the field of conjecture of the prehistorian. They cannot tell us what our own past *was*, because this is unknowable in all but a trivial sense.

In the penultimate chapter ("The Purpose of Avebury") the author boldly gives his view, with suitable cautions, about the funerary and fertility rituals for which Avebury *may* have been built. Here, and indeed throughout the book, he displays the vision of a creative artist, in putting flesh and colour upon the bare bones of the archaeological evidence, no less pitifully exiguous here than in the other stone circles that he has studied. Amongst contemporary writers on prehistory he has the rare and enviable gift of evoking for the mind's eye the past-which-might-have-



Standing Stones at Avebury

Photo: M. A. Summerfield

been, without ever departing so far from the raw evidence as to strain the credence of the reader.

This admirable but discursive recension raises many questions about what is still unknown. Some of these can and should be answered, and soon, as far as available techniques allow.

In particular, the chronology of Avebury is still guess-work. On page 166 Burl gives a table of dates which divides the building of Avebury into three phases (interior stone circles; earthwork and main circle; avenues). This is plausible on the evidence available; but it is based wholly on the dating by analogy of pottery types, which is notoriously uncertain. The surviving finds from excavations can surely yield a suitable series for radiocarbon dating. Could not this be undertaken by the Department of the Environment, the official guardians of the monument?

Likewise, the former positions of the missing stones in the North and South circles, and in the eastern half of the great circle inside the ditch, still await identification. So do the stone-holes and buried stones of the Kennet Avenue, beyond the part excavated by Keiller and Piggott, and almost the whole of Stukeley's Beckhampton Avenue to the south-west. The latter, long regarded as the illusory product of Stukeley's perfervid imagination, has now been restored to credibility by recent finds. Further confirmation is needed, if the full extent of the Avebury complex is to be understood.

To search by excavation would be premature, because all excavation destroys more evidence, unrecognised, than it yields to the most skilled, experienced and conscientious excavator. The state of the art has not advanced so far beyond the work of the 1930s as to justify further knowing destruction.

There are, however, techniques of geophysical survey which can be applied, some of them developed specifically by the Ancient Monuments Laboratory of the Department of the Environment. There is a case here for a long-term programme of research, in which the diagnostic use of the simple hand-held probe should not be neglected. It is one of the defects of this book that these future problems are not spelled out, with non-destructive solutions (or at least programmes) of this kind.

There are other defects too. The values used by the author (page 175) in calculating the man-hours required for the building of the Avebury earthwork are grossly in error; but the corrections are largely self-cancelling, so that the result is still about 1.5 million man-hours. His second estimate (pages 175–76), which is about half of the first, is invalid because it is based on the raw evidence from the experimental earthwork on Overton Down, and makes no allowance for the scale-factor required. This is included in my own empirical formula on which the first estimate is founded.

Furthermore, although the neighbouring Neolithic causewayed camp on Windmill Hill, and the West Kennet Long Barrow, are discussed and interpreted in detail in the text, no plan is given of either. Worse still, there is no modern plan of Avebury itself, with the stones numbered, although individual stones are often referred to by their numbers in the text. These omissions mar what would otherwise be a quite excellent book. □

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Boswellian enthusiasm for science

M. Rowan-Robinson

Broca's Brain: Reflections on the Romance of Science. By Carl Sagan. Pp.347. (Random House: New York; Hodder and Stoughton: London, UK, 1979.) $12.95; £6.95.

By his clear and witty writing and by his willingness to write about those eternally popular subjects, the planets and life on other worlds, Carl Sagan has captured a large audience. His last book *The Dragons of Eden,* on the evolution of human intelligence, won him a Pulitzer Prize, and the present book is already high on the American bestseller list. Sagan is a real phenomenon and we have to take him seriously as a major populariser of science today.

Broca's Brain opens with a superb evocation of the inner recesses of the Musée de l'Homme in Paris, where Sagan finds and muses on the preserved brain of Paul Broca, a pioneer of neurosurgery and anthropology in the mid-nineteenth century. This could almost be the opening of a novel in the manner of Balzac. The themes of the book are "the joys and social consequences of the scientific endeavor; borderline and pop science; the not entirely different subject of religious doctrine; the exploration of the planets and the search for extraterrestial life; and Albert Einstein . . .". The disparate nature of these themes is matched by an almost complete absence of continuity between the chapters of the book. This is a collection of lectures which Sagan has given on various occasions and of articles that have appeared in magazines as diverse as *Scientific American* on the one hand and *Playboy* and *TV Guide* on the other.

Although it is disappointing that these themes are not developed in the coherent and sustained way that we would expect from a purpose-written book, these essays are lively and entertaining, bubbling with amusing anecdotes about science and scientists. Sagan would make an excellent scientific Boswell. I particularly liked "A planet named George", about how the planets, satellites, and their craters and mountains, have come to have their exotic names. Not many people will be aware that the satellites of Jupiter are named after the illicit loves of his Greek counterpart, Zeus. Sagan feels it is rather unfair that Zeus' wife Hera does not get a mention. There is a marvellous, absurd picture of the international astronomical committee which decides these matters worrying about whether the Third World is sufficiently represented in the names of craters and asteroids and deciding that maybe some other creative humans besides astronomers and astronomers' wives ought to be so honoured.

However, Sagan aspires more to the role of Johnson, the moralist. He has a rather uncritical enthusiasm for science and technology and deplores the "sparse, unimaginative and ineffective efforts at science education" of American media and schools. He sees the best protection against abuses of science as a scientifically literate populace. "In exchange for freedom of inquiry, scientists are obliged to explain their work". But Sagan, while apparently admiring the anti-militaristic activities of Einstein and Russell, does not face up to the issue of military research, that all the most dangerous research (nuclear weapons, germ warfare and so on) is controlled by governments in an atmosphere of rigid secrecy.

There are a series of essays on pseudoscience and fringe science: astral projection, spirit rapping, precognitive dreams, UFOs, Velikovskianism, and so on. There is a particularly good essay on the problem of the remarkable astronomical knowledge of the Dogon



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people of Mali. As Sagan points out, their astronomical knowledge is just not remarkable enough to have been learnt from little green men in UFOs and could all have been learnt from contacts with Europeans. Sagan has performed a useful service here in collecting together these examples of public gullibility and demonstrating how a mixture of deliberate fraud and uncritical thinking has allowed millions to be bamboozled throughout history.

Yet when later in the book Sagan turns to discuss religion, his own critical apparatus seems to be switched off. "These mystical insights must mean something . . . If religions are fundamentally silly, why is it that so many people believe in them?" — an argument which could equally be applied to belief in UFOs or astral projection. Sagan's excitement by the phenomenon of mystical visions, and his curious attempt in the last chapter of the book to link these and all religious ideas to experiences associated with birth, suggest that Sagan is about to become a "born-again" believer in — something.

Although Sagan is rightly caustic about some of the absurdities of the popular pseudosciences he makes no effort ot discuss the problem of demarcation between science and pseudoscience, which has so occupied the attention of Karl Popper and his followers. Sagan's championing of science seems positively naive at times. Compared with the doctrines of pseudoscience ". . . science is more intricate and subtle, reveals a much richer universe and powerfully evokes our sense of wonder. And it has the additional and important virtue — to whatever extent the word has any meaning — of being true". Astrologers would obviously be happy with this statement with the word "astrology" substituted for "science". That is not what distinguishes science from pseudoscience.

On the exploration of the Solar System, Sagan is naturally interesting and thorough, for this is his own area of expertise. It would be interesting some day to hear what it is like for a scientist to work on one of these NASA missions. I find it rather touching that Sagan's belief in the existence of life elsewhere in the Universe is so passionate that he cannot bring himself to mention NASA's official interpretation of the Viking Lander mission to Mars, namely that Mars is chemically but not biologically active.

In comparing Sagan with popularisers of astronomy like Hoyle or those grand old rivals Eddington and Jeans, it has to be said that he has so far set himself an easier task. Those earlier masters set out to popularise the astronomical research of their times: they were ahead of the tastes of their public and tried to take their readers with them into the world of the modern astronomer. Carl Sagan, like the efforvescent Patrick Moore, talks for the most part about the areas already popular with the public: the Solar System, space travel, life in the Universe. Sagan's references to the "new" astronomies, radio, infrared, X-ray, and so on, seem to me to be perfunctory. He is not even particularly good on a topic which might be expected to have appealed to him, astronomy from space platforms. He refers to "an astronomical telescope in earth orbit called Copernicus", without mentioning that it is primarily the ultraviolet band that the instruments on the Copernicus satellite have opened up. He seems to think that X-ray emission from gas in clusters of galaxies was discovered by the recent HEAO satellites, whereas this was one of the achievements of the pioneering Uhuru satellite launched in 1970.

Considering that most of this material has been seen or heard by the public before, there are a couple of minor errors that ought to have been removed by now. It is not the orientation in space of a molecule that is quantised (page 17) but its angular momentum or energy. And galaxies do not disappear from view over the event horizon (page 296): on the contrary they swim into view over it. And as for the suggestion that psychedelic molecules exist in great abundance in plants because of artificial selection as a result of cultivation by human beings. . .

But these reservations do not diminish my enjoyment of the book. *Broca's Brain* is an entertaining collection of essays by a very good popular-science writer. □

Michael Rowan-Robinson is Reader in Astronomy at Queen Mary College, University of London, UK.

In the conflux of eternities

Freeman J. Dyson

In the Centre of Immensities. By Bernard Lovell. Pp. 174. (Hutchinson: London; Harper and Row: New York, 1979.) £5.50.

LOVELL'S life-work has been the exploration of the Universe by means of radio-telescopes. He was one of the small group of pioneers who understood thirty years ago that the new science of radio-astronomy would give us a wider and deeper view of the Universe than optical observations alone could provide. The achievements of the telescopes which he built have proved his vision right. In our modern theories of the structure and development of the Universe, the evidence obtained from radio observations plays a central and indispensable role.

In this book Lovell is not concerned with his own work as an observer. He is describing the historical growth of our understanding of the size and shape of the Universe and of our own situation within it. He puts this astronomical history within the context of the wider philosophical and religious struggles which have raged around the growth of modern science. He believes, in opposition to the prevailing fashion of scientific positivism, that the creative interaction between astronomy, philosophy and religion is not yet at an end. His purpose is to demonstrate that the discoveries of the past fifty years have confirmed rather than contradicted the words spoken by the mathematician-philosopher Whitehead in 1925: "When we consider what religion is for mankind, and what science is, it is no exaggeration to say that the future course of history depends upon the decision of this generation as to the relations between them."

To readers moderately conversant with the facts of astronomy, Lovell's expository chapters describing the growth of astronomical knowledge will be easy to follow but not particularly novel. On the technical level, his exposition does no differ substantially from the accounts given in other popular books such as Steven Weinberg's *The First Three Minutes* (André Deutsch/Fontana: London; Basic Books: New York; for review, see *Nature*, **267**, 291; 1977). The exciting things in Lovell's book are non-technical. His unique quality as a thinker is displayed in the philosophical and ethical discussions with which the book begins and ends, and in the breadth of his knowledge of history and literature. Chapter 1 starts with Saint Thomas Aquinas and ends with Thomas Carlyle, both of them great writers and profound thinkers, both of them synthesisers who tried to reconcile scientific truth with religious vision, both of them destined to be pushed aside by a younger generation of impatient fact-finders.

Lovell takes his title from Thomas Carlyle's "Sartor Resartus". Man, according to Carlyle, "sees and fashions for himself a Universe, with azure Starry Spaces, and long Thousands of Years. . . as it were, swathed in, and inextricably over-shrouded; yet it is sky-woven and worthy of a God. Stands he not thereby in the centre of Immensities, in the conflux of Eternities?" If Lovell's book succeeds in persuading a new generation of readers to look at the Universe through the eyes of Thomas Carlyle, it will have done us all a great service. □

Freeman J. Dyson is Professor of Physics at the Institute for Advanced Study, Princeton, New Jersey.

How physicists understand matter

Gerald Feinberg

The Forces of Nature. By P. C. W. Davies. Pp. 231. (Cambridge University Press: Cambridge and New York, 1979.) Hardback £12; paperback £4.25.

PAUL DAVIES is a mathematical physicist with both interest and talent in writing about modern physics for non-specialists, His previous works in this genre include *Space and Time in the Modern Universe* and *The Physics of Time Asymmetry.* There he treated aspects of relativity, thermodynamics and comology. In his present volume, *The Forces of Nature,* Davies turns to the central concern of twentieth-century physics, the structure of matter, and the interactions (forces) acting among subatomic particles. The book uses relatively little mathematics, and, in most places, requires no previous knowledge of physics on the readers' part. The result is a broad and usually clear survey of the ways in which physicists now understand matter. However, *The Forces of Nature* is missing a certain spark that was present in Davies' previous books. Also, it is somewhat marred by a number of inaccurate or misleading statements. These do not distort the main thrust of what Davies is doing, but they do make his treatment of some subjects unreliable. As the book does not try to present original contributions to physics, it is style, clarity and accuracy that are of greatest importance, and I will concentrate this review on these questions.

As might be expected from an author whose own research is highly mathematical, *The Forces of Nature* is very much a theorist's book. The ideas of physics are described in detail, but the experimental methods that led to these ideas are given perfunctory treatment. This is especially true of experiments carried out over the past thirty years. I cannot help thinking that this disparity in the treatment of theory and experiment will give readers a distorted view of how physics has developed in the past, and how it may proceed in the future.

The Forces of Nature begins with a brief introduction to Newtonian mechanics, and to electrical, magnetic and gravitational forces. The discussion of the forces is very clear and precise, but that of mechanics is less so. In particular, the description of energy is so vague that a reader will have trouble knowing what the concept means. This introductory chapter concludes with a section on special relativity theory.

In his next chapter, Davies outlines the quantum theory of atomic structure as developed between 1900 and 1930. The essential feature of such an exposition is its treatment of quantum mechanics, perhaps the major intellectual achievement of the twentieth century. Davies approaches quantum mechanics through its wave aspect, which he explains very well, using analogies with musical instruments. He uses this discussion to indicate why electrons in an atom have discrete energy levels. However, the correct way of describing electrons in an atom, through the notion of a quantum state characterised by definite values of energy, angular momentum and spin, is not introduced clearly. As a result, Davies' discussion of the Pauli exclusion principle is vaguer than it need be. Furthermore, the distinction between fermions and bosons is described in such a way that one of the great achievements of quantum field theory, the connection between spin and statistics, seems a tautology. These problems could have been avoided with only a little more care.

Davies turns next to nuclear structure, and discusses the constituents of nuclei, nuclear stability, and alpha and beta radioactivity. These subjects, especially nuclear stability, are treated well. The description of beta radioactivity, with its distinction between neutrinos emitted in electron capture and antineutrinos emitted in neutron decay, leads into a discussion of antiparticles in general. Here Davies uses the Dirac hole theory, with its heavy reliance on the exclusion principle. This approach has some virtue in making the existence of positrons plausible, but it has two decisive disadvantages. One, mentioned as an afterthought by Davies, is that the hole theory does not apply to bosons, such as charged pions, which also have antiparticles. The other is that the hole theory obscures the fundamental symmetry between particles and antiparticles by making antiparticles seem like interlopers in a Universe composed of particles. That picture is, however, false, and the observed surplus of electrons over positrons, and of protons over antiprotons, is actually an empirical fact that needs explanation, which physicists are beginning to find in the early Universe. Although the hole theory did lead Dirac to predict antiparticles, it has probably outlived its usefulness and should be retired to the same honoured resting place for outmoded physical concepts as Maxwell's mechanical models of electromagnetic fields.

The notion of antiparticles next leads Davies to consider particle creation and annihilation, one of the more surprising consequences of the union of quantum mechanics and relativity. Davies approaches this through the use of quantum field theory, again skillfully using analogies with the behaviour of musical instruments. An emphasis on the field, rather than the particle aspect of relativistic quantum theory is not the rule among physicists today, most of whom think of quantised fields as no more than convenient mathematical tools for the description of interacting particles. Davies has an additional motive in emphasising fields, which is the desire to demystify the abrupt, unanalysable creations and destructions of particles, by treating these processes as more familiar changes in field amplitudes. However, the amplitudes of the fields are also quantised, and also change abruptly when the corresponding particles are created or destroyed. It is therefore no more accurate to think of transitions between states of a quantised field in classical terms than it is to think of transitions bestween particle states in that way. In both cases, transitions occur in a way that cannot be predicted in advance, although the transition can be measured after it does occur. It is not a difference here that impels us to use quantised fields. Davies does not mention recent work on solitons, and on vacuum excitations, which is a more basic effort to make the field aspect of relativistic quantum theory play a role separate from the particle aspect.

One of the important consequences of quantum field theory is the notion of virtual particles, and their effect on the properties of 'real' particles. Any existing configuration of particles spends part of its time in other states, which may consist of any combination of particles with the same value of conserved quantities, such a charge, as the original configuration. Davies gives a mostly clear exposition of this idea, both for the electromagnetic field, and for the fields associated with pi mesons. Along the way, he introduces the Feynman diagrams that are now universally used by physicists to symbolise various creation and annihilation processes. However, Davies does overstate the significance of virtual particles in one respect, when he says that the real particles produced in various collision processes are simply the virtual particles already present in the projectile and target. This picture is only true for special cases, such as the emission of very low energy photons in a collision between charges. It is not true for the production of high energy particles in collisions, in which case some of the emerging particles must be imagined to have been created in the collision process itself. William Blake's vision of "a World in a grain of sand" may be qualitatively satisfied by the existence of virtual particles, but it should not be taken as quantitatively sound.

The subject of the next section of *The Forces of Nature* is the profusion of subatomic particles discovered in recent years, and the phenomena of strong and weak interactions that they undergo. This description is fairly thorough and easy to

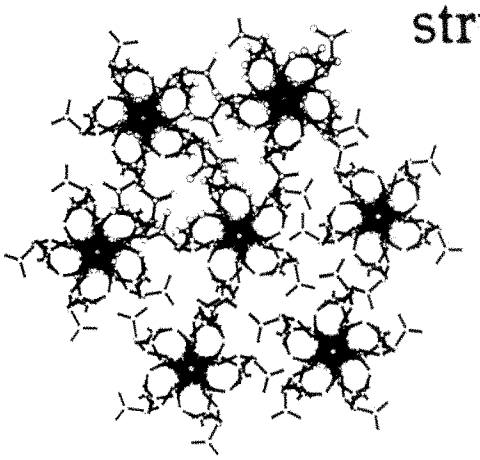
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follow. One important part of the theory of subatomic particles treated in this section is the set of symmetry principles that these interactions satisfy. Davies' discussion of the basis of these principles is curiously Aristotelian, relying on the invariance properties of empty space. Although he is able to draw correct conclusions from this analysis, past experience should make us cautious about the extent to which such a style of argument can be trusted. It would be just as easy to infer Aristotle's form of inertia as Galileo's form from Davies' arguments.

The final section of *The Forces of Nature* deals with recent developments, such as the quark theory of hadrons, and the unified theories of weak and electromagnetic interactions. The discussion here is very up-to-date, and includes the discovery of the upsilon meson, and of parity non-conservation in deep inelastic electron scattering. There are also nice discussions of spontaneous symmetry breaking and of some reasons why quarks may not be observable. Once again, some things in the text are misstated, such as the mechanism by which charged pion decay occurs in the quark model.

A general stylistic objection to the book is that over and again Davies resorts to the phrase "it can be shown," or the like, a device that will probably annoy others as much as it does this reviewer. But on the whole, *The Forcers of Nature* is a considerable achievement in popular science writing. It is most likely to appeal to readers with an abstract turn of mind, who like deductive reconstructions of physical phenomena. □

Gerald Feinberg is a Professor of Physics at Columbia University, New York.

Einstein's ideals

Eugene Wigner

Albert Einstein, The Human Side: New Glimpses from His Archives. Selected and edited by Helen Dukas and Banesh Hoffman. Pp.168. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) $10; £4.95.

THE purpose of this book is to acquaint the reader with Einstein's social ideas, with his emotional life, with his convictions concerning the objectives man should strive for. And he surely abided by the ideals he put forward in his letters as well as any person could. The book is almost completely restricted to these aspects and contains hardly any information about Einstein's scientific accomplishments. It deals with what the authors call the human side.

As far as Einstein's own direct contributions to the book are concerned, these are almost completely restricted to letters which he sent out and a few which he drafted but did not send off. It does contain also two vitae — one written when he was 17, the other when he was a member of the Prussian Academy of Sciences, in Berlin at the age of 53 — and these are interesting to compare. Most of the rest of the book is due to two of his admirers: his almost life-long secretary Helen Dukas and his past friend and collaborator Banesh Hoffmann. They call themselves Selectors and Editors of the letter collection, but their contribution is actually much greater. It includes also the description of Einstein's relationship to the addressee of the letter, Einstein's attitude towards him or her, the motives leading Einstein to write the letter and the circumstances under which it was written. All this is done very clearly. They have also written a very nice introduction to the book.

Most of Einstein's letters were originally written in German and some were sent in that language. Others were drafted in German then translated into English by Einstein himself, but in some cases only the original German draft is available. In both these cases translations have been made by the Selectors and Editors, and in some cases this has been a difficult task. A particularly difficult case is that of the poems written by Einstein for inclusion in the letter. But whenever the letter's English formulation was not by Einstein himself, the German original is reproduced in the second part of the book ("German Originals", pp. 119-163). In a few cases, one must admit, the translation is less lively than the original, but it is good even in these cases.

The book does give the reader a vivid impression of the breadth of Einstein's interests, of his modesty and desire to help his fellow men and please them, of his convictions about what is good and what is evil. As to the first question, even the writer of this review, though he knew Einstein since about 1925, was not aware of all his interests. Besides all branches of physics, it also included music — his favourite composers were Bach and Mozart but he did have an affectionate regard for some of the later ones. His love of his violin was well-known to all his acquaintances. But in addition to these well-known interests, he showed great fondness towards the great philosophers of the past, and many of his letters are enlivened by philosophical remarks which give pleasure to the interested reader of the book and must have given even more pleasure to the original receipient of the letter. "The concept of a soul without a body seems to me empty". "What I see in Nature is a magnificent structure that we

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can comprehend only very imperfectly, and that must fill a thinking person with a feeling of humility" (pp 39 and 40) are two such passages. But there are many, and even if one does not fully agree with them, all are refreshing. He also defended philosophy in general against its deprecators even when recognising its weakness: "Philosophy is like a mother who gave birth and endowed all other sciences" (p106) is perhaps an exaggeration but a well-meant one.

Einstein's love of poesie is also little recognised. He even wrote some poems, all of a slightly humorous nature, for his letters. About ten of these are contained in the book, and all are amusing.

Einstein's attitude towards religion is also clearly expressed in his letters. He felt — as many people do today — that its main role should be in the ethical domain, whereas its cognitive assertions should be more in the background. He also writes a good deal about the extinction of life — one must, with sadness, come to the conclusion that he enjoyed his last few years very little and considered the extinction of his life not only cognitively, but to some degree also emotionally, as a liberation from obligations.

Einstein's political convictions are also very apparent from his letters. These are, of course, generally known: he was opposed to all dictatorships, be they of the national socialistic — that is, Hitler-inspired and anti-semitic — or of communistic nature. In fact, one draft letter (pp110-112) is so vehement that it was not sent out but, according to the introduction preceding it, "must have brought him a feeling of relief".

The preceding discussion contains, of course, only a superficial review of the kind of information the reader will gain about Einstein's interests and thinking when reading this book. And the information will appear in a much more lively fashion than the foregoing summary presents it.

The book contains more than a hundred letters. To whom were they addressed? A few to his sister, Maja, a few more to his earliest physicist friends Zangger, Ehrenfest and Lanczos, but even more, about eight, to his psychiatrist friend Juliusburger. There is no letter to either of his wives or to the rest of his family. The rest of the letters are practically all answers to letters he received, each from a different person. With about half of these he had some acquaintance, the other's being written to please or comfort people he had not heard of before. Many of these letters, the writing of which was stimulated by kindness or compassion, contain passages which are truly memorable, and manifest deep insights. A few of the other letters are directed to editors of journals and about four to ministers of churches. These latter all have a strong political undertone.

It is not clear how the letters published were selected, nor is it clear how Einstein had chosen the letters to be answered. In one of his letters (p43) he praises the one which he answers as being very reasonable, in contrast to the hundreds of other letters which he was receiving, most of which, of course, he did not answer. It would also be interesting to know where and how the letters and the drafts of the letters published were found.

The English language part of the book (the first 115 pages) are arranged into 12 sections. These sections, however, have no titles and it is not clear on what basis the various letters are distributed into these sections. This has advantages if one reads the book from cover to cover — it is not easy to digest 10 philosophical letters in succession and not refreshing to read several complaints about politicians' misdeeds one after the other. But if one wants to recover a passage which one remembers vaguely, it would be good to have either a definite order of the articles in the book, or at least an index at the end of it. Similarly, the time ordering of the letters seems to be a random one. Thus, Section 6 (pp54-74) has articles dating from '33, then '51 and '53, then '27, again '33, '35, '21, and so on, ending up with one of '27. The subjects vary equally greatly, the first is political, the next a thank-you for the offer of financial help, the next refers to physics, then comes a letter to a student publication, then a praise of science in general and so it goes on. It is amusing to read all this, but a catalogue surely would be useful.

After all this criticism, let me compliment again the Selectors and Editors for the very good job they have done. It surely required not only knowledge but also devotion to the job and a true interest in the human side of Einstein. □

Eugene Wigner first met Einstein in 1925, while a student in Berlin. He had closer contact with him after 1933 when both lived in Princeton, New Jersey.

The quantum and its supporting planks

P.T. Landsberg

Black-Body Theory and the Quantum Discontinuity, 1894-1912. By T.S. Kuhn. Pp. 512. (Clarendon/Oxford University Press: Oxford and New York, 1978.) £12.

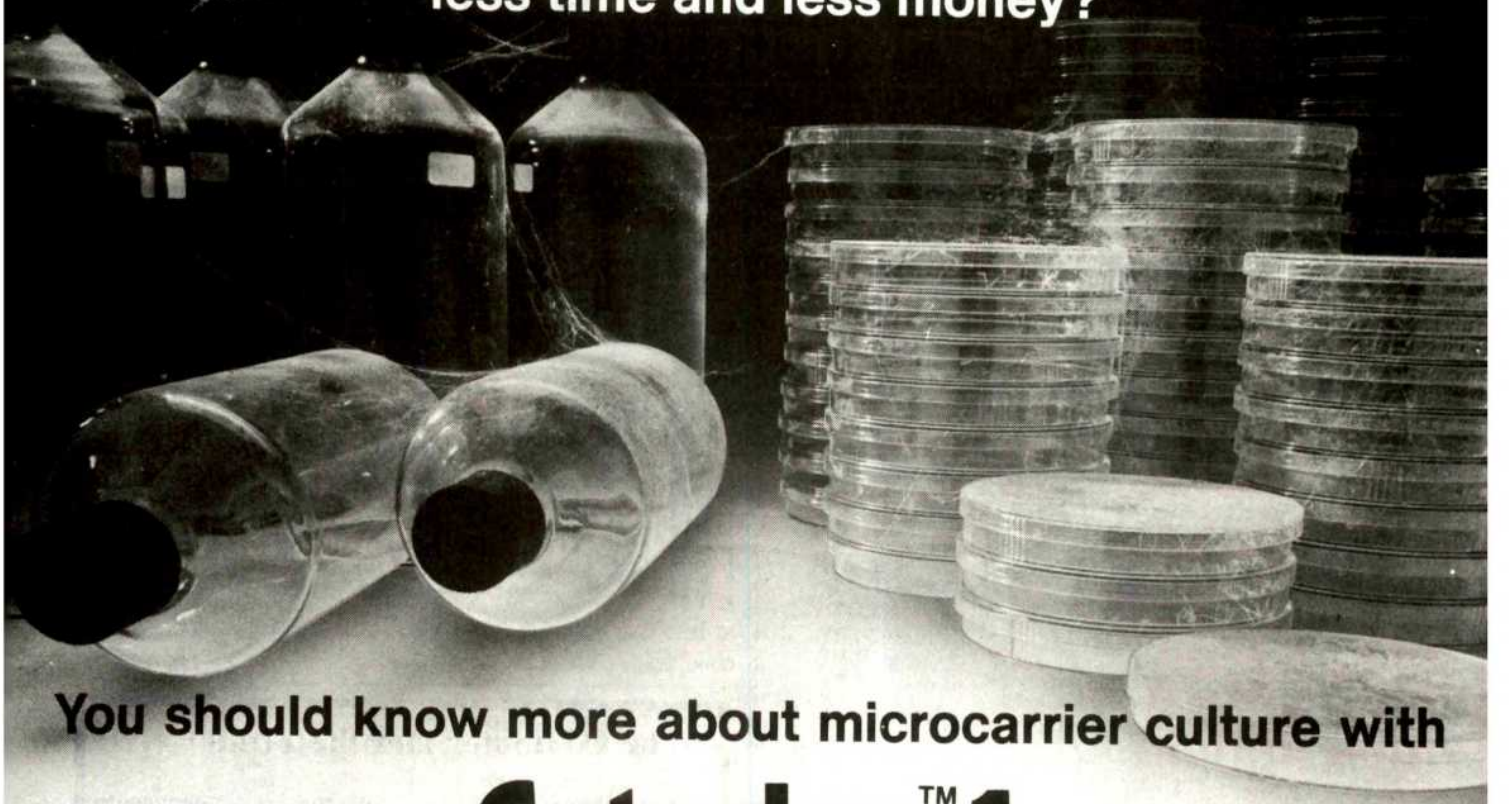
THE realisation that discontinuities (or quanta of energy) are needed for the description of microscopic phenomena represented one of the great transformations of human thought. Not that everything was believed to be continuous before that — the atomic concept of Democritus, the distinct notes of musical instruments, comets and explosions had been part of the intellectual furniture for centuries, and they certainly implied discontinuities of sorts. But they were not fundamental. Comets continued to be, when not in view; explosions could presumably be analysed into a more or less continuous sequence of chemical reactions; musical notes could be changed by altering the tension in the wire; and the constitution of atoms (if they existed, one would add in 1900) posed in any case intractable problems, for example, how could they be indivisible if they participated in the production of spectral lines? Indeed, as W.K. Clifford observed, an atom must be at least as complicated as a grand piano. Upon this placid scene, in which physics seemed to be approaching a completed structure, there floated gently (for it did not 'burst') Planck's theory of the energy density, as a function of frequency, of radiation when in thermal equilibrium with bodies at temperature T (black-body radiation). Shortly afterwards arose the problems of relativity, specific heat anomolies, etc., and so black-body radiation never lost the charisma of having been the first problem area which brought deep changes to classical physics. It did so by suggesting that an atom oscillating linearly at a frequency ν has available only discrete energies $h\nu$, $2h\nu$, . . . and so on, whereas classical theory would have allowed a continuous range of energies. Here h is the newly introduced constant of nature, now called Planck's constant.

At precisely what time and through whose pen did these discontinuities enter physics? The normal answer is that the year was 1900 and the man was Max Planck. In this scholarly study, which can boast 90 pages of notes and references, Kuhn suggests an amendment. Planck restricted the energy of N oscillators to be integral multiples of an energy unit, ε say, a type of "quantisation" already envisaged by Boltzmann in 1877, but he did not impose this constraint *on a single oscillator* until after 1906 (see p126), the year in which this requirement had been emphasised independently by Paul Ehrenfest and Einstein, "the two physicists who first recognised that Planck's black-body law could not be derived without restricting resonator energy to integral multiples of h or some equivalent non-classical step" (page ix).

In the Epilogue Kuhn discusses Planck's "second theory" of heat radiation (1911) which introduced the notion that the mean energy of an atomic oscillator goes to a constant and non-zero value at the lowest temperatures. Although this zero-point energy remained important, Planck's second theory was

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also to suffer profound amendments. By now, as Kuhn shows, the scientific community was turning to the quantum theory of specific heats.

Scientific community? In those far off days of the first Davis Cup matches, of Henry Ford and Thomas Mann, the number of authors who wrote on black-body theory did not exceed 20 even by 1914. By that time there were still only about sixty-five authors who wrote on the quantum theory, but the number was rapidly rising (as clear from the only three figures in the book). Does today's science harbour another growth point of this significance so that mathematically simple but conceptually gigantic leaps can again be made? Those who doubt that such a growth point can still be found in physics hope, like drowning men clutching at a straw, that it is perhaps still possible in biology. It is, however, a delight of science that there could yet be hope for physics. Who knows?

Mathematics is not popular with readers of book reviews, but to understand the background of Planck's arguments a gentle sniff of three results is needed. First, as black-body radiation does not depend on the material systems with which it is in contact (Kirchhoff, 1860), the simplest systems can be chosen. These are oscillators, N in number say for each frequency ν. The mean energy $U(\nu, T)$ of one of them is then simply related to the energy density $\varrho\ (\nu, T)$ of the radiation (Planck, 1899). Secondly, thermodynamics tells us how to find the mean entropy $S(U)$ of an oscillator in terms of U, provided $U(\nu, T)$ is known. (Example: if $U = kT$, then $dS/dU = 1/T = k/U$, so $S = k\ln U$). Thirdly, if one has a fundamental model to calculate the number W of so-called microstates of a system one can evaluate the entropy with the aid of a simple relation (Boltzmann 1877).

Now a remark about three Planck papers. In March 1900 he injected an empirical formula for ϱ due to Wien (1896) into this theoretical structure, and went from ϱ to U to S. But then he could only check certain properties of S and nothing revolutionary resulted.. In October 1900 he injected into the same structure a new (S, U)-relationship which contained the classical result $d^2S/dU^2 = -k/U^2$ and the Wien result $d^2S/dU^2 = -A/U$ as limits. It was a trial, or interpolation formula, and, passing in the opposite direction now, he inferred U and ϱ. The latter result was what has since become known as the famous Planck distribution law. "Permit me to direct your attention to this new formula" says Planck, "... I consider it to be the simplest apart from Wien's." But it had no firm basis in theory! Planck tells us that he worked very hard to find such a basis and on 14 December 1900, he produced a W-expression from the mechanics of oscillators. This is where he was very brief and not very clear. In any case, he now proceeded in the accepted logical direction from W to S to U and ϱ, and that was the essential job done. Or was it?

The problem for the studious reader, 75 years later, is: how did he get W? He says about the total energy of the N oscillators (we suppress the suffix ν, as we consider a given frequency), that "it is necessary" that it shall be considered to be "not a continuous, infinitely divisible quantity", but that "it shall be considered to be discrete and to consist of a finite number of equal magnitudes" (*Ann. Phys. 2*, 4,553; January, 1901), "which is the most essential point of the whole calculation" (*Verh. d. Dtsch. Phys, Ges.*, **2**, 237; December, 1900).

Kuhn unfortunately does not quote these passages, and argues that the fact that the total N-oscillator energy E_N is an integer n multiplied by an energy unit ε does not give the essence of the discontinuity: "If quantization is the subdivision of total energy into finite parts, then Boltzmann is its author" (p127). this is a possible point of view. An alternative, more generous, interpretation is to recall, first, that the oscillators are assumed far enough apart not to interact with each other. Hence, if one adds a single oscillator, then its average energy $E_1 = U$ must be the difference between the two Planckian results $E_{N+1} - E_N = n\varepsilon - m\varepsilon$ where n and m are integers. Hence Planck's result extends to $U = r\varepsilon$ $(r = 1, 2, \ldots)$. Secondly, what was still a fictitious model for Boltzmann, who after all took the limits $\varepsilon \to N \to \infty$, had become physically relevant in Planck's hands. Nevertheless, it is certainly true that Planck's reticence is puzzling, as has been pointed out (for example, by M.J. Klein, *Paul Ehrenfest*, Vol I, p228, North-Holland: Amsterdam, 1970; by H.-G. Schöpf, *Von Kirchoff bis Planck*, p106, Akademie: Berlin, 1978). Although there will therefore be discussions among historians of science sparked off by this book, we shall never know exactly what was in Planck's mind in 1900, nor is it very important to know. It strikes me that Planck knew the chain ϱ - U - S - W very well and had great confidence in his ϱ; so he worked backwards to find the W needed. Perhaps it was because Boltzmann had done much of the mathematics needed by Planck that Planck did not expound the theory of the W as clearly as he might have done. That there existed a sensible expression at all was to him, and rightly, an achievement. To infer single oscillator quantisation (which he might have done by the argument given above) was in 1900 against his wish, as he did not want to leave classical physics, as Kuhn reminds us again so clearly. In any case should an *average* oscillator energy be quantised? Surely not, a point apparently missed by some later commentators.

As to Einstein, he emerges as hardly ever wrong, as the following story shows. On 12 June 1913, Planck, Nernst,

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Max Planck awarding the Planck Medal to Albert Einstein in 1929

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Rubens and Warburg proposed Einstein for full membership of the Prussian Academy of Sciences, describing the achievements of the 34-year-old scientist in glowing terms. From the point of view of the book under review it is of interest that they did, however, remark: "That he has occasionally missed the target in his speculations, as for instance in his light quantum hypothesis, is not something that should count too severely against him; for even in the most exact of sciences it is not possible to introduce real advances without occasionally taking a risk". One can almost hear Planck's voice. It is a sobering thought for those called upon to judge the work of their peers in scientific academies, research committees, book reviews and promotion boards that this distinguished group of four was so devastatingly wrong; after all the light quantum hypothesis was essential to Einstein's explanation of the photoelectric effect which was to be cited by the Nobel Committee in 1921. This interesting matter did not escape Kuhn's eagle eye (though he attributes it to 1914) but he notes it only in passing (p182). Yet there it is. The careful and conservative Planck held back. Friend and supporter of Einstein's though he was - to the extent of risking the concentration camp by his 1933 declaration in support of Einstein - the photon picture of radiation was too much in advance of current thinking in 1905.

My main criticism of this fine book — so lovingly researched as to include abstracts, reviews and relevant citations of some of the key papers — is that this very detail has prevented a tight organisation of the material and of the author's conclusions, which are therefore somewhat buried at various points, in spite of a good index. The Epilogue might have included a two- or three-page forward look into the 1920s, for reasons which will be touched on below.

An alert reader may ask a question, to which the book does not supply an answer, as to whether systems other than oscillators, for example rotators, were studied in contact with black-body radiation. If not, why not? In any case, the element of confusion between E_N and E_1 and between quantising $E_1 = U$ as against quantising the unaveraged single oscillator energy, means that the Planck argument has to be replaced. By treating radiation as a gas of "light quanta" and introducing the notion of indistinguishability of quanta of given energy, Bose (1924) gave an argument which no longer depended on any parameters of the material system in contact with the radiation. In a modern exposition Bose's W had the same form as Planck's, $W = (N + P - 1)!/P!(N - 1)!$. However, Planck oscillator number N became the number of states in a typical narrow energy range of the quanta considered, and Planck's integer $P = E_N/\varepsilon$ became the number of quanta to be distributed over these states in an optimal manner. (For Planck P was the number of discrete energy elements to be distributed over the N oscillators). Although this was much more satisfactory, the result was approximate (Stirling's approximation). On the whole this does not worry people. However, one cannot conceal that this approximation breaks down for the small number of quanta which can be found in high energies, and I found (1954) that if the Stirling approximation is improved one gets into trouble. The Planck law *can* be derived as an exact result, but as far as I know only by using the grand canonical ensemble or by taking a limit.

Two queries may be in order in conclusion. In "overblowing" a wind instrument the note jumps as a higher mode is excited. This is typical of *classical* discontinuities. They are not mentioned in the book but it would be of interest to study them, together with Schrödinger's and Planck's approaches, in a historical context. Secondly, the case studied here suggests (though one must not generalise from one example) that a great scientific advance — a revolution if you like — tends to dissolve into little steps on closer inspection. The same man may take different steps at different times, so that he may in the end have made a major contribution; but small steps they are nevertheless. If this is a general rule, with a few exceptions such as general relativity, it would be interesting to hear Kuhn, the author of the *Structure of Scientific Revolutions* (1962) consider it in the light of this latest book. The topic itself has been much discussed of course (for example, by S. Toulmin, *Human Understanding*, Vol. 1, 1972), but it is particularly interesting in connection with the story of black-body radiation. □

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articles

Direct imaging of the twin QSOs 0957+561 A and B: the gravitational lens interpretation

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Recent observations have indicated that the twin quasistellar objects 0957+561 A and B might be images produced by a gravitational lens. New direct observations are presented, including the probable detection of an object between the two quasistellar objects. The implications of these observations for the nature of the twin quasistellar objects are discussed.

WALSH *ET AL.*[1] have recently announced the discovery of a pair of quasistellar objects (QSOs) that are separated by 5.7 arc s and that have the same emission redshift, 1.405. These QSOs, 0957+561 A and B, were found to have several similarities. The redshifts of the C IV λ1,549 and C III] λ1,909 emission lines are significantly different in each QSO but identical to the accuracy of measurement between the two objects. The equivalent widths of these two lines are, respectively, the same in the two QSOs. Furthermore, a strong low-Δz absorption system at approximately the same redshift was seen in the spectrum of each object. Subsequent spectroscopic observations at the Multiple Mirror Telescope by Weymann *et al.*[2] revealed that the redshifts of the absorption systems in the two QSOs differed by 7 ± 15 km s^{-1}. The amazing similarities between these two objects have led to speculations[1,2] that the image of a single QSO was being split by a gravitational lens in the line of sight, and to crude calculations of the parameters necessary to produce such an effect.

The large mass implied by these calculations ($\sim 10^{13} M_{\odot}$) led us to believe that if such an object existed and was a particularly massive galaxy, it should be visible on a suitably deep, direct picture of the region. Also, because the images formed by a gravitational lens are not expected to be circular, it might be instructive to examine the size and shape of the images of the QSOs in such a picture.

Observations

The two QSOs were observed on 15 June 1979 with the Kitt Peak National Observatory (KPNO) 4-m telescope and the ISIT video camera. Four 13.6-min. integrations were obtained through an *R* filter. The resulting field in this configuration is ~75 arc s on a side; each pixel is ~0.29 arc s. The seeing was estimated at the telescope to be 2 arc s. Basic reduction of the data included subtraction of a dark frame and division of the object field by a flat-field exposure obtained through the same filter.

These four frames were inspected using the KPNO interactive picture processing system (IPPS). This inspection revealed that the quality of the images in the last exposure was significantly poorer than in the other three due to deterioration of the seeing at high air mass (sec $z \sim 2.2$).

In each of the first three frames the centre of gravity of each QSO image was determined. These centres agree to ≤1 pixel frame-to-frame. An equal weight average of these three exposures was then calculated and used for all subsequent measurements. This final picture is shown in Fig. 1.

Analysis

Several different types of measurements were made using the IPPS surface brightness routines written by Strom[3].

Simulated aperture photometry of the two QSOs in our averaged frame provides a scale factor for conversion of our arbitrary intensity units into *R* magnitudes. The adopted *R* magnitudes for the two QSOs were taken from Walsh *et al.*[1]. The determined relationship is $m_R = -2.5 \log I + 30.6$, where I is the total intensity within the aperture. Magnitudes obtained in this manner are probably accurate to ±0.5.

We attempted to detect or set an upper limit to any excess flux present between the two QSOs. First, an average value for the sky was determined by sampling several blank areas adjacent to the QSOs. A sky surface brightness of 20.5 mag arc s^{-2} in *R* was measured. Next, the contribution of each QSO to the point midway between them was determined. The flux in a series of 1.16 arc s circular apertures, arranged as shown in Fig. 2, was measured. The radius of each semicircle is equal to half the separation of the two QSOs. This procedure was used to estimate the possible error due to asymmetric images.

This possibility has important implications for the significance of a detection. If we find that the standard deviation of the measurements among the apertures is less than or equal to that expected from measurement uncertainties (for example, photon

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statistics, flat-field and dark-count uncertainties, nonlinearity in the detector), we can justify using the standard deviation of the mean as an estimate of the accuracy. If, however, the variation among the apertures around the QSOs is larger than that expected from measurement uncertainties, we must conclude that our apertures are sampling a variety of values, and in this case the standard deviation of a single aperture is a better estimate of the accuracy.

Unfortunately, the results of this comparison are somewhat ambiguous. Measurement uncertainties were determined by sampling the sky in a series of adjacent apertures, yielding a variation of ~1%. Around QSO A, the standard deviation of the measurements exceeds that expected by ~45%. A χ^2 test indicates a 15% probability of a distribution as unfavourable as that observed arising from the expected gaussian distribution. Around QSO B, the standard deviation is almost twice that predicted by measurement errors. The variation in this case, however, seems to be systematic in that the four apertures most nearly along the line perpendicular to the separation of the two QSOs are much higher than the other four. The most likely explanation of this asymmetry is that we have marginally resolved QSO B in the perpendicular direction.

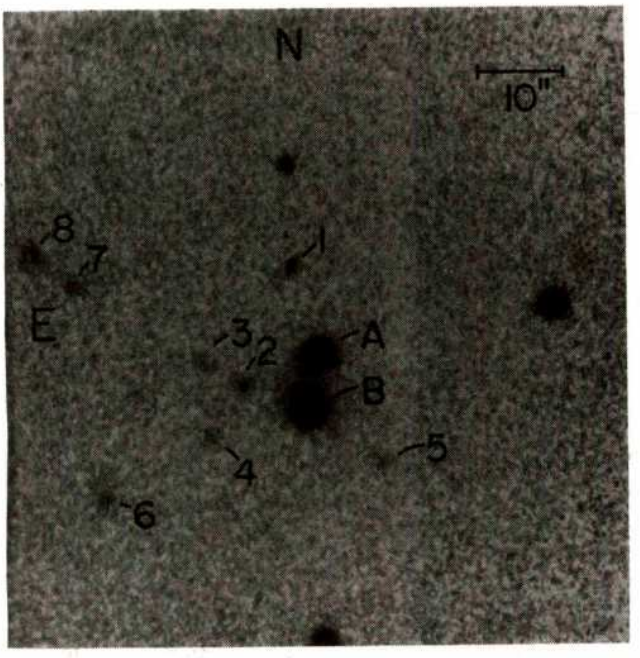


Fig. 1 The final averaged R-band picture of the region surrounding 0957+561 A and B. The eight galaxies listed in Table 2 are indicated.

Because of the difficulty in ascertaining which types of errors dominate, we will consider two cases. The flux measurements for each case are presented in Table 1. In case 1 we consider the variation around QSO A to be statistical. For QSO B, we use only the four apertures most nearly along the line of separation and consider the variation among them to be statistical. In case 2 we consider all variations to be real and, therefore, use all points in the mean; the quoted errors are the standard deviation of a single measurement. Case 1 indicates a 5.4 σ detection which corresponds to $m_R = 23.1$; case 2 indicates a 2.0 σ detection which corresponds to $m_R = 23.4$.

Visual examination of our data suggested that the image of QSO B is larger than that of QSO A. To quantify this we measured the radial profiles of the two QSOs perpendicular to their separation. Since the central surface brightness of QSO B is ~12% lower than that of QSO A, we scaled up the measurements of QSO B by this amount before comparing them with QSO A. The resulting profiles (Fig. 3) clearly show that at low surface brightness QSO B is ~10% broader than QSO A.

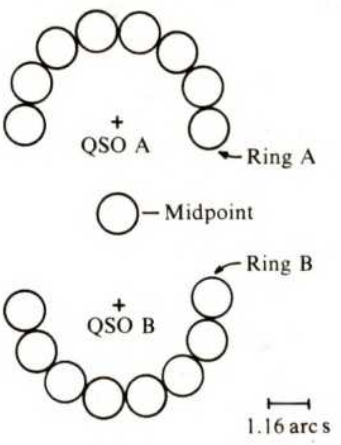


Fig. 2. The geometric configuration used in the photometric measurements.

We measured the magnitudes of eight faint nonstellar objects in the field. These measurements were obtained through (1) a 1.16-arc s aperture for comparison with our midpoint photometry; and (2) a 6.44-arc s aperture which should contain essentially all the light of each galaxy (Table 2). The galaxy designations are shown in Fig. 1.

Discussion

Two ways of qualitatively accounting for the original observations have been proposed. The first hypothesis is that the two objects are separate QSOs that, by chance or environment, have evolved with remarkably similar characteristics. This hypothesis requires that almost identical absorbing clouds are found in the line of sight to both QSOs at precisely the same redshift. The second interpretation involves a single QSO whose image has been split by a gravitational lens.

To discuss the gravitational lens hypothesis more quantitatively, we begin by calculating the expected characteristics of the lens object. This calculation involves the following three assumptions. (1) The object is a galaxy with a velocity dispersion given by

$$[\sigma\ (\mathrm{km\ s^{-1}})]^2 = \frac{106{,}875\ (1+z_L)\,[(1+z_Q)^2-1]}{[(1+z_Q)^2-(1+z_L)^2]} \qquad (1)$$

where z_L is the redshift of the lens, z_Q is the redshift of the QSO. This formula is derived from equation (1) of Weyman *et al.*[2] using their values of θ_{sep} and n. The mass is derived from this by assuming that the galaxy is an isothermal sphere.

Table 1 Excess flux measurements

	Case 1 Measurement error	Case 2 Real variation
Ring A	798.0±66.6	798.0±188.5
Ring B	1,115.9±62.8	1,360.9±291.5
Midpoint	2,904.1±158.3	2,904.1±158.3
Excess	990.2±184.7	745.2±382.0

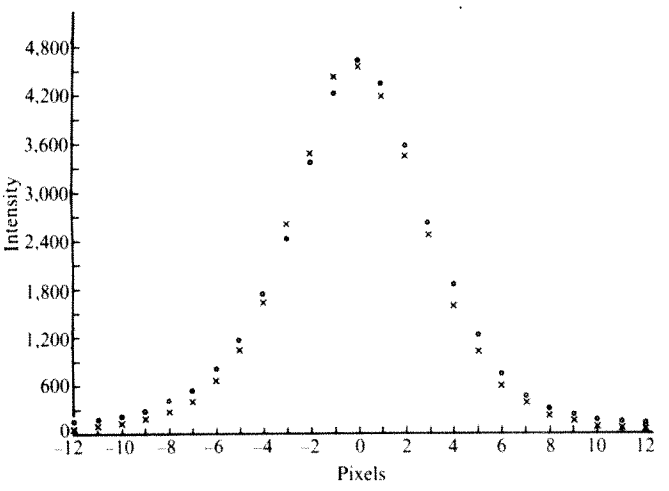


Fig. 3 Profiles of the two QSOs (×, QSO A; ○, QSO B) in the direction perpendicular to their separation. The intensities have been normalised to the central three pixels of QSO A.

(2) As the mass-to-light ratios M/L for galaxies are poorly determined, we have done the calculation for two cases: $M/L = 5$ and $M/L = 30$ in solar units.

(3) $H_0 = 50$ km s^{-1} Mpc^{-1} and $q_0 = 0$. Table 3 lists, for various possible z_L values, the absolute R magnitude of the lens galaxy, M_R, the total apparent R magnitude of the lens galaxy, m_T, and the expected R magnitude through a 1.16-arc s diameter aperture m_{ap}. R-band K corrections were extrapolated from the published V-band K corrections of Pence[4]. The magnitude difference between m_T and m_{ap} was calculated from the M87 surface photometry of Young *et al.*[5] by comparing the flux in the small aperture with that in a 6 arc s aperture at each redshift. The resulting magnitudes in Table 3 are very uncertain, primarily due to the K corrections and luminosity evolution which we have ignored. Moreover, comparison of our measured magnitude with the numbers in Table 3 is hindered by uncertainty in the absolute calibration and ignorance of the correct M/L. Thus differences of up to 1 mag in this comparison are within the possible range of error.

The possible interpretations of our detection of an $m_R = 23.1$ object between the two QSO images are: (1) the object could be the lens galaxy; (2) the object could be a faint field galaxy in the line of sight; (3) the object could be the galaxy responsible for the absorption system.

If the detected object is the lens galaxy, it can be seen from Table 3 that it must be located at very nearly the QSO redshift, or it must have a large M/L. The extended profile of QSO B may argue for the validity of the lens interpretation. If the lens is circular and slightly out of the line of sight, it is expected that the two images will not be identical. The image further from the lens will be brighter and more elongated perpendicular to the separation of the images[6]. Note that QSO B has a redder continuum than QSO A[1]. If this is indicative of greater extinction, then QSO B is the brighter of the two.

The other two interpretations of the nature of the object between the QSO images argue for the 'non-lens' picture. The explanation that the object is a field galaxy located by chance in the line of sight is unlikely because of the probability of finding a galaxy brighter than 23.1 mag in a 1.16-arc s aperture. Using the faint galaxy counts of Tyson and Jarvis[7], we estimate that the likelihood of this is $<10^{-3}$. These counts and the work of Kron[8] indicate that the expected number of galaxies visible in our field is ~3–5, and thus it is unlikely that the eight galaxies seen are associated with a rich cluster.

The third possibility, that the object is the galaxy responsible for the absorption in the two QSOs 65 kpc apart, cannot be ruled out by this statistical argument. However, the primary argument against this explanation is the very large luminosity required for the absorbing galaxy. Even in the optimistic M/L case, the necessary luminosity requires that $M_V < -26$. This seems unlikely, considering the local galaxy luminosity function from which it seems that significant luminosity evolution would be needed to attain such a value. The evidence at this time[7,8] favours little, if any, luminosity evolution at this magnitude. Also, no QSO with a redshift as large as 1.4 has been detected as nonstellar.

Table 2 Field galaxy photometry

No.	m_R (aperture 1.16 arc s)	m_R (aperture 6.44 arc s)
1	22.4	20.9
2	22.6	20.5
3	24.0	22.1
4	23.4	21.3
5	23.5	22.1
6	22.9	20.6
7	22.9	20.8
8	22.3	20.1

Table 3 Calculated lens characteristics

	$M/L = 5$			$M/L = 30$		
z	$-M_R$	m_T	m_{ap}	$-M_R$	m_T	m_{ap}
0.1	23.2	15.9	17.5	21.3	17.8	19.4
0.2	24.1	16.7	18.0	22.1	18.7	20.0
0.4	24.9	17.9	18.8	22.9	19.9	20.8
0.6	25.4	19.2	19.9	23.5	21.0	21.7
0.8	26.0	20.0	20.7	24.0	22.0	22.7
1.0	26.6	20.4	21.0	24.6	22.4	23.0
1.2	27.4	20.7	21.3	25.5	22.6	23.2

Conclusions

We have obtained a deep R-band picture of the double QSO 0957+561 A and B. We have detected excess flux between the two QSOs which corresponds to $m_R \simeq 23.1$. The significance of this detection strongly depends on the nature of the errors. QSO B was found to have a more extended radial profile than QSO A in the direction perpendicular to their separation.

Both these results can be interpreted as consistent with the two competing hypotheses: a gravitational lens or two distinct QSOs. However, a statistical argument favours the gravitational lens interpretation.

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Oxidation of aerosoled ultrafine iron particles

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Aerosoled ultrafine iron particles, in the few hundred ångstrom size range, have been subjected to different oxidation modes. X-ray and Mössbauer techniques have been used to study the morphology of the oxide scales and the diminution of the recoilless fractions and the hyperfine fields.

ULTRAFINE particles of iron are of considerable interest as they form the active component of magnetic tapes now under development by the recording industry. A way of making small metal particles is by an aerosol or evaporation technique developed more than a decade ago[1-3]. In essence, metals are evaporated in an inert gas atmosphere at reduced pressure. The metallic atoms have a reduced mean-free-path, and eventually condense to form small particles. Particle size can be controlled to some extent by changing the pressure or species of the inert gas.

Very small iron particles are pyrophoric. The oxidation of metallic iron in the form of sheets, foils, or even single crystals has been studied extensively[4-7]; the motivation has been an interest in corrosion and passivation processes. Little is known about the oxide layers formed on the surface of ultrafine iron particles[8-10]; it is therefore timely and relevant to investigate the oxidation modes for such particles.

Earlier experiments in our laboratory showed the oxide layer to be composed of small crystallites with the spinel structure[10], probably a mixture of Fe_3O_4 and γ-Fe_2O_3. Further, for a sample with particles an average diameter of 238 Å, only a slight Mössbauer absorption was observed at room temperature, whereas at 77 K a well-defined hyperfine-split spectrum was obtained[11]. Apparently at 300 K most of the particles experience a recoil on absorption of the γ-ray photon. The Debye–Waller factor for small particles has attracted the attention of many researchers, and does not yet seem to be settled[12-18].

Iron particles can be passivated by an O_2 and N_2 gas mixture low in oxygen content[19], that is, by a slow oxidation. At the other extreme, on exposure to open air even at room temperature, the ultrafine particles glow, indicating that a relatively high temperature is reached. Hence, the chemical and physical properties of ultrafine particles may be different, depending on whether a slow or a fast oxidation is used. This article reports on an X-ray and Mössbauer study of aerosoled iron particles subjected to different extreme oxidation modes. Special attention is given to the morphology of the oxide, the recoilless fraction, and the hyperfine fields.

Oxidation experiments

Five samples of small iron particles in the 200–400 Å range were prepared by evaporation into a 1 to 3 torr helium gas atmosphere. They were produced at Osaka University, Japan, and made available through Professor A. Tasaki and Dr M. Takao. The samples labelled A–E were collected in sample containers, and sealed in a helium gas atmosphere.

The oxidation of the samples was achieved in the following way. Sample E was immediately exposed to air, and after completion of the rapid oxidation was quenched and stored in ethyl alcohol. Sample D was suddenly exposed to air about 3 weeks later; it glowed and the powder colour changed rapidly from black to dark brown. Thus, samples D and E were subjected to fast oxidation. Samples A, B and C were oxidised slowly by air entering through a small leak in the seal. Although this slow leak continued over a period of a few weeks, the actual oxidation rates are of course unknown. When samples A, B and C were exposed to air, no further changes occurred, indicating that complete passivation had taken place, as suggested by Tasaki *et al.*[20]. Indeed, X-ray and Mössbauer data showed that another sample, similar to C, underwent no measurable changes over a 3-year period.

The average particle size of each sample was determined by the Brunauer–Emmett–Teller (BET) nitrogen adsorption method assuming a spherical shape. The results are listed in Table 1.

Table 1 Properties of samples A, B, C, D and E

Sample	Particle size (Å)	Crystallite size (Å)* Fe	Crystallite size (Å)* Oxide	Composition (%)† Fe	Composition (%)† Oxide	Mössbauer absorption 300 K
A	380	133	68	18	82	Almost none
B	238	76	51	5	95	A little
C	293	100	27	20	80	A little
D	329		101		≈100	Hyperfine split
E	366		120		≈100	Hyperfine split

* Determined from X-ray line broadening using (311) for cubic oxide and (110) for metallic Fe.
† Obtained from Mössbauer absorption area.

X-ray measurements

X-ray diffractograms were taken for the five samples using CoKα radiation; part of the recorder plots is shown in Fig. 1. For slowly oxidised samples (A, B, C) the diffraction profiles indicate two phases—one with the crystal structure of ordinary α-Fe, and the other, constituting a significant fraction, has the structure of spinel iron oxide, either magnetite (Fe_3O_4), γ-Fe_2O_3 or a mixture of both oxides. No line corresponding to α-Fe_2O_3 (haematite) was detected. Although the superlattice lines peculiar to γ-Fe_2O_3 were not observed, the presence of γ-Fe_2O_3 is not ruled out for the following reasons. First, as shown in our earlier study[21], the transformation from Fe_3O_4 to γ-Fe_2O_3 takes place with good probability in the small particle region. Second, the vacancy-ordered superstructure lines associated with bulk γ-Fe_2O_3 are reduced or absent in ultrafine particles because then the vacancies, although they exclusively occupy B sites, are

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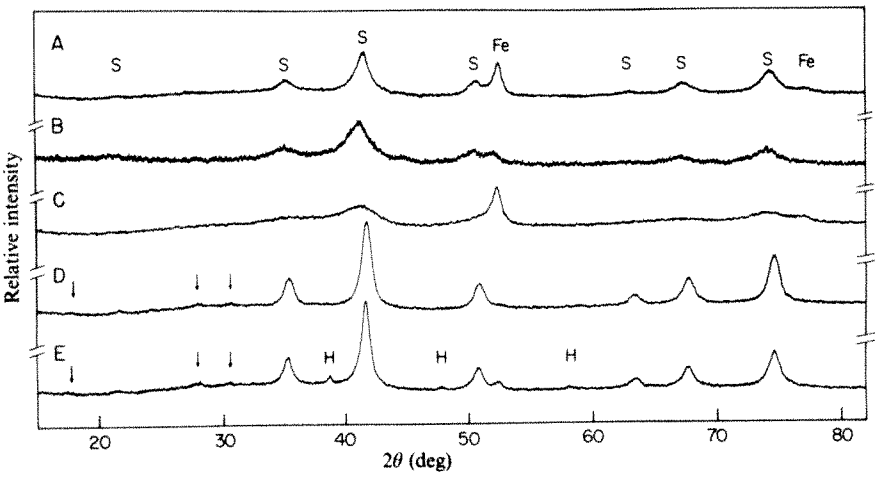


Fig. 1 X-ray diffraction recorder plots for samples A, B, C, D and E. Here 2θ is twice the Bragg angle. S, spinel type iron-oxide phase; Fe, Fe phase; H, α-Fe_2O_3 phase; arrow, vacancy-ordered superlattice line peculiar to γ-Fe_2O_3.

disordered[22]. Third, every oxide diffraction line for the three samples is slightly shifted to higher angles, that is, towards the γ-Fe_2O_3 and away from the Fe_3O_4 positions. Therefore, the data probably imply that the oxide is a mixture of stoichiometric Fe_3O_4 and γ-Fe_2O_3.

Sample D, oxidised by the fast mode, does exhibit faint vacancy-ordered superlattice X-ray lines. No line of metallic iron or α-Fe_2O_3 was detected. In addition, both the X-ray line positions and the dark brownish colour are close to those associated with γ-Fe_2O_3. It follows that this sample is almost entirely composed of γ-Fe_2O_3.

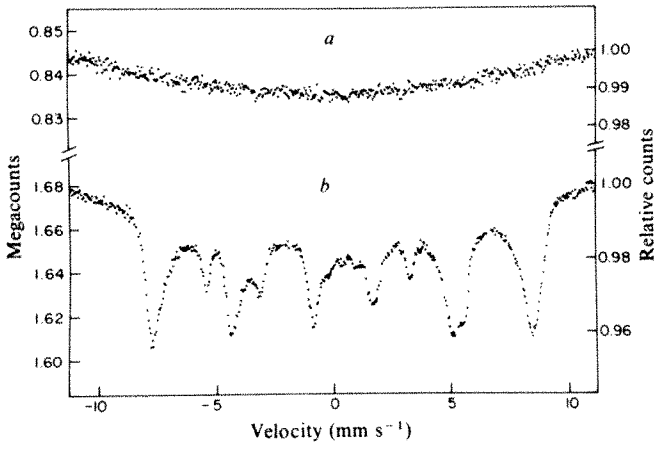


Fig. 2 Mössbauer spectra for aerosoled Fe particles subject to slow oxidation, sample A (380 Å). *a*, At 300 K; *b*, at 77 K.

The pattern for sample E, oxidised quickly then quenched in C_2H_5OH, shows γ-Fe_2O_3 lines, including superlattice ones, as well as low intensity α-Fe_2O_3 and α-Fe lines. The presence of the latter suggests that the oxidation may have been faster for sample E than D, and that the quenching may have been a factor.

The variation in the X-ray linewidths is of particular interest. For samples A, B and C, the spinel lines are much broader than those of iron. In addition, the spinel linewidths of these three samples are significantly different. Even in the slow-oxidation mode, physical and chemical processes may depend delicately on the actual oxidation rate. These observations imply a considerable difference in the iron-oxide crystallite size for the A, B and C samples. Further, the iron-oxide layer is apparently not continuous, but consists of very fine crystallites. A similar observation was made by Boggs *et al.*[23] in a microscopic study of the oxidation process for iron.

Although the lines for samples D and E are much sharper than for A, B and C, they, nevertheless, are still relatively broad. The average crystallite sizes for the oxide and the metallic iron were determined for all samples using the Scherrer relation (Table 1). The faint superlattice lines of samples D and E, observed in spite of the relatively small average crystallite size, seems to imply that some of the crystallites are large enough to have developed vacancy ordering.

To generalise, the X-ray studies imply that on slow oxidation of ultrafine aerosoled iron particles only partial oxidation occurs with the formation of finely divided oxide crystallites on the iron. On the other hand, on fast oxidation, all the iron is oxidised into crystallites that are relatively large.

Mössbauer studies

Mössbauer measurements were made on the five samples at 77 and 300 K; some spectra are shown in Figs 2, 3 and 4. To make an absorber, the powder was dispersed in acetone, dried in air, and pressed between two plates of Perspex or beryllium. The source was ^{57}Co in a chromium host. The spectrometer was conventional, and all but one of the spectra (Fig. 3*b*) were taken with a fly-back drive.

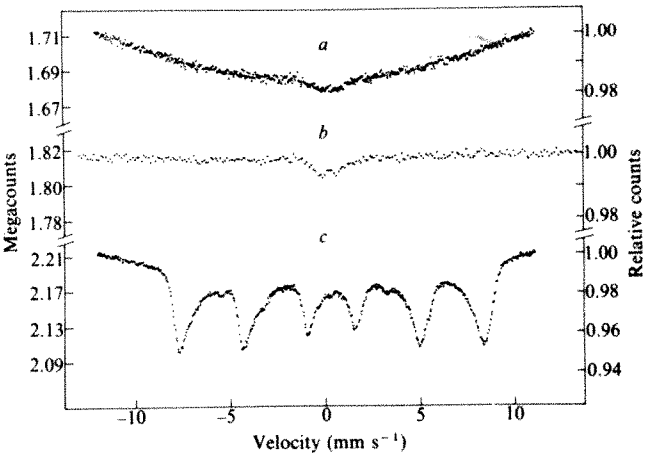


Fig. 3 Mössbauer spectra for aerosoled Fe particles subject to slow oxidation, sample B (238 Å). *a*, At 300 K; *b*, at 300 K, taken with a triangular waveform drive and folded; *c*, at 77 K.

Mössbauer spectra for samples A and B, both subjected to the slow-oxidation mode, are shown in Figs 2 and 3 respectively. An interesting and important feature is the lack of absorption at room temperature. Note that fine particles, close to or less than 100 Å in diameter but prepared by other methods, such as chemical precipitation, do display a central superparamagnetic absorption sometimes accompanied by hyperfine split patterns at $T = 300$ K. Therefore, the implication is that a majority of the ^{57}Fe nuclei in the aerosoled particles experience a recoil on absorption of the γ ray. Perhaps the iron atoms or ions are loosely bound within an aerosol particle, and further, any inter-particle interactions are relatively small. A weaker than normal binding would lead to a reduction in the Debye–Waller factor.

As the parabolic-shaped background introduces some ambiguity, a spectrum for sample B at 300 K was obtained with a triangular-shaped drive and a greater velocity range, and folded; it is shown as Fig. 3*b*. The small but non-zero central absorption shows that although the number of recoilless absorptions was

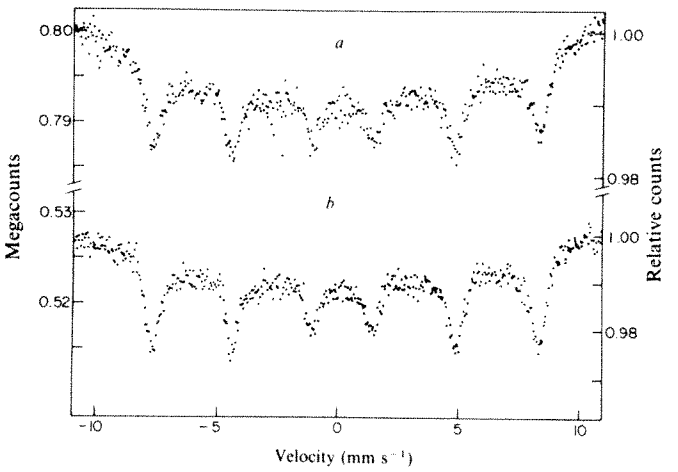


Fig. 4 Mössbauer spectra for aerosoled Fe particles subject to fast oxidation. *a*, Sample D (329 Å) at 300 K; *b*, sample E (366 Å) at 300 K.

small, the crystallites were in the superparamagnetic size range. This observation is fully compatible with the X-ray results.

In contrast, samples D and E, subjected to the fast-oxidation mode, exhibit hyperfine-split Mössbauer spectra at 300 K with, however, some reduction in the recoilless fraction as compared to bulk materials (Fig. 4*a*, *b*). When the temperature is elevated during the non-isothermal oxidation, not only do the oxide crystallites grow larger, but apparently the quality of crystallinity is also improved. Better developed crystallites would be expected to have strengthened atomic or ionic binding, and therefore a Debye temperature closer to that for the bulk.

In some situations, the Mössbauer effect can be used for phase analysis. By referring to standard spectra of α-Fe and the various iron oxides, the relative amounts of these phases can in principle be determined. By referring to Figs 2*b* and 3*c*, it is clear that both iron and iron-oxide phases are present. An earlier Mössbauer analysis of sample C established that the oxide is partially oxidised Fe_3O_4, possibly a mixture of stoichiometric Fe_3O_4 and γ-Fe_2O_3 (ref. 10). A similar situation seems to apply for samples A and B. The oxidised fractions of samples, A, B and C were determined from the 77 K spectra by comparing the areas of the oxide and metallic iron patterns employing computer fitting and assuming equal recoilless fractions for the two phases (Table 1). For samples D and E, the Mössbauer spectra confirm the X-ray analysis that the particles are almost entirely γ-Fe_2O_3.

Table 2 Hyperfine field at 77 K for aerosoled iron particles subject to slow oxidation

Sample	Hyperfine field (kOe)			Linewidth (mm s^{-1})		
	Fe	Inner sextet	Outer sextet	Fe	Inner sextet	Outer sextet
A	243	470	507	0.400	0.994	0.645
B	347	465	503	0.493	1.179	0.673
C	344	451	494	0.492	1.285	0.773
Micrometre-sized γ-Fe_2O_3*		508	525		0.562	0.533

One six-line pattern is assumed for metallic Fe and two six-line patterns are assumed for cubic iron-oxide.
* From ref. 25.

The hyperfine fields will now be discussed. In order to reduce the complexity of the analysis, the 77 K spectra of samples A, B and C were fitted with one six-line pattern for the iron and two six-line patterns for the iron oxides. This procedure is an over-simplification as the oxide is probably a mixture of γ-Fe_2O_3 and Fe_3O_4 in the Verwey state. The hyperfine fields and linewidths obtained are listed in Table 2. The iron hyperfine fields are close to the normal, bulk values, although the lines are somewhat broadened. However, the oxide's hyperfine fields are 30 to 50 kOe lower than those for micrometer-sized γ-Fe_2O_3 particles at 77 K. As a particle is made up of a central iron core covered with finely divided oxide crystallites, the magnetic moments of the oxide crystallites experience a considerable interaction at the iron interface. Then, the tendency towards superparamagnetic behaviour may be largely quenched at 77 K. However, fluctuations in the oxide's magnetisation direction may still be taking place, and if so, would lead to a reduction of the hyperfine field. This hypothesis is a modification of a collective magnetic excitation model proposed by Mørup and Topsøe[24]. In other words, the diminished value of the hyperfine field is an intrinsic size effect complicated by the exchange interaction at the iron interface.

Thus the oxidation mode used with ultrafine aerosoled iron particles profoundly influences the morphology of the oxide scales and the recoilless (Mössbauer) fraction. Some magnetic properties of these particles can therefore be controlled by the conditions of oxidation, and this feature should be of great utility in small iron particle applications.

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A different genetic code in human mitochondria

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Comparison of the human mitochrondrial DNA sequence of the cytochrome oxidase subunit II gene and the sequence of the corresponding beef heart protein shows that UGA is used as a tryptophan codon and not as a termination codon and suggests that AUA may be a methionine and not an isoleucine codon. The cytochrome oxidase II gene is contiguous at its 5' end with a tRNA^{Asp} gene and there are only 25 bases at its 3' end before a tRNA^{Lys} gene. These tRNAs are different from all other known tRNA sequences.

THE genetic code has been considered to be universal since the early work on *Escherichia coli* and mutant eukaryotic proteins. Once the code was established it was argued that any change would be lethal. This view was substantiated by the development of rapid RNA and DNA sequencing methods which have explored a wide variety of gene sequences that could be related to their protein sequences. We present evidence here, however, that mitochondria have an altered genetic code. This conclusion is based on the nucleotide sequence of a region of human mitochondrial DNA which was shown to code for an amino acid sequence homologous to the known protein sequence of the cytochrome oxidase subunit II (COII) of bovine mitochondria[1]. The homology was such that it was concluded that this region of the DNA was coding for the human COII gene. However, there were three TGA codons in the reading frame that was coding for the protein. This demonstrated that UGA was not acting as a termination codon. Moreover, the amino acid present in the bovine protein in all positions where TGA occurred in the human DNA sequence was tryptophan. Such a correspondence could only be satisfactorily explained if UGA was coding for tryptophan rather than for termination. Similar evidence is also presented that suggests that AUA is a methionine codon and not isoleucine.

Human mitochondria have a separate genetic system with a DNA genome of molecular weight 10×10^6. Figure 1 shows the organisation of the human mitochondria genome[2,3] aligned with the *Mbo*I restriction enzyme map. HeLa cell mitochondrial DNA (mtDNA) has been shown by hybridisation to code for a 12S and a 16S ribosomal RNA and at least 19 tRNAs[2-4]. HeLa cell mitochondrial translation products are less well defined and only cytochrome oxidase subunits I, II and III have been identified so far[5]. By analogy with other mitochondria systems (for review see ref. 6) genes for four proteins of the ATPase complex and one of the cytochrome bc_1 complex should also be present. However, it has become apparent recently that more proteins (at least 19) are coded for by HeLa cell mtDNA[7]. Both strands of HeLa cell mtDNA are completely transcribed symmetrically[8,9]. The rRNAs, the majority of the rRNAs and all but one of the polyadenylated RNAs hybridise to the heavy strand (H strand)[2,4,8,10]. Thus, the light strand (L strand) is defined as the main coding strand, having the same sequence as the rRNAs, tRNAs and mRNAs.

In the sequence of the COII gene region presented here we have identified two putative tRNA genes flanking the COII gene. A tRNA^{Asp} gene is 'butt-jointed' to the initiation codon of the COII gene, that is, there is no intercistronic space between them, and there are only 25 nucleotides between the termination codon of the COII gene and a tRNA^{Lys} gene. These tRNA genes are unusual in that they differ considerably in the 'D loop' and 'TψC loop' from all other known tRNA sequences.

DNA sequence analysis

*Mbo*I restriction enzyme fragments of human mtDNA obtained from a single term placenta have been cloned in the *Bam*HI site of pBR322 (ref. 42). Sequence analysis of clones pmt 110, 140 and 101, containing *Mbo*I fragments 6a, 15 and 6b, respectively (see Fig. 1), was by the chain termination method of Sanger *et al.*[11]. Single-stranded template DNA was prepared by the exonuclease III method of Smith[12]. By cleaving the duplex circular DNA at either the unique pBR322 *Hin*dIII site or the unique pBR322 *Sal*I site and digesting with exonuclease III, either strand of the region containing the inserted DNA was obtained pure for the primed synthesis DNA sequencing reaction. Small restriction fragment primers were prepared both from the pBR322 sequences flanking the insert, so as to prime into the insert from either side, and from within the insert.

The sequence determined is shown in Fig. 2. Sequence analysis of the *Alu*I restriction fragment spanning *Mbo*I fragment 15 which was cloned separately shows that there are no small *Mbo*I fragments between *Mbo*I fragments 6a, 15 and 6b (A. R. Coulson, personal communication). Two examples of chain termination sequencing gels are shown in Fig. 3. Figure 3*a* shows the sequence from −120 through the tRNA^{Asp} gene and

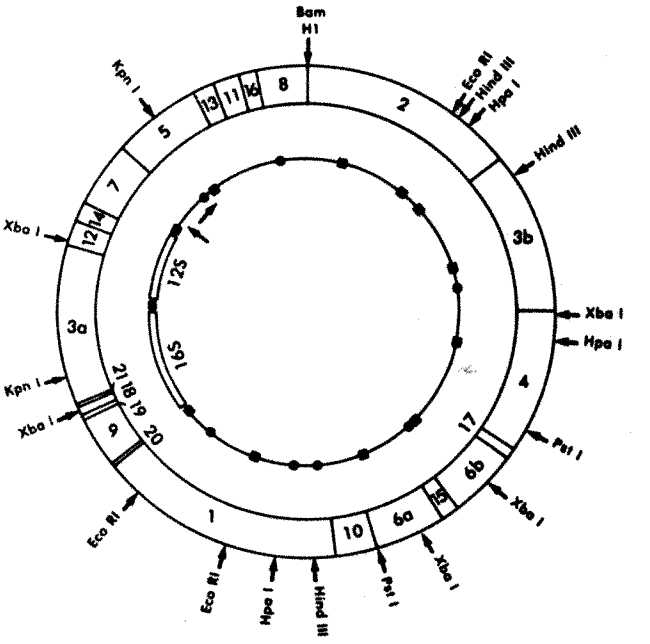


Fig. 1 The genetic map of the human mtDNA genome[2,3] is aligned with the *Mbo*I restriction enzyme map[42]. The precise order of fragments 21, 18 and 19 is not known. The sites of cleavage of some other enzymes are also shown[43,44]. The origin and direction of H-strand synthesis is shown[43,44]. The L-strand sequence 5' to 3' is anticlockwise and codes for the 12S and 16S rRNAs and tRNAs marked ■ (complementary to H strand). The H strand codes for the tRNAs marked ● (complementary to L strand). The COII gene is centred approximately on *Mbo*I fragment 15.

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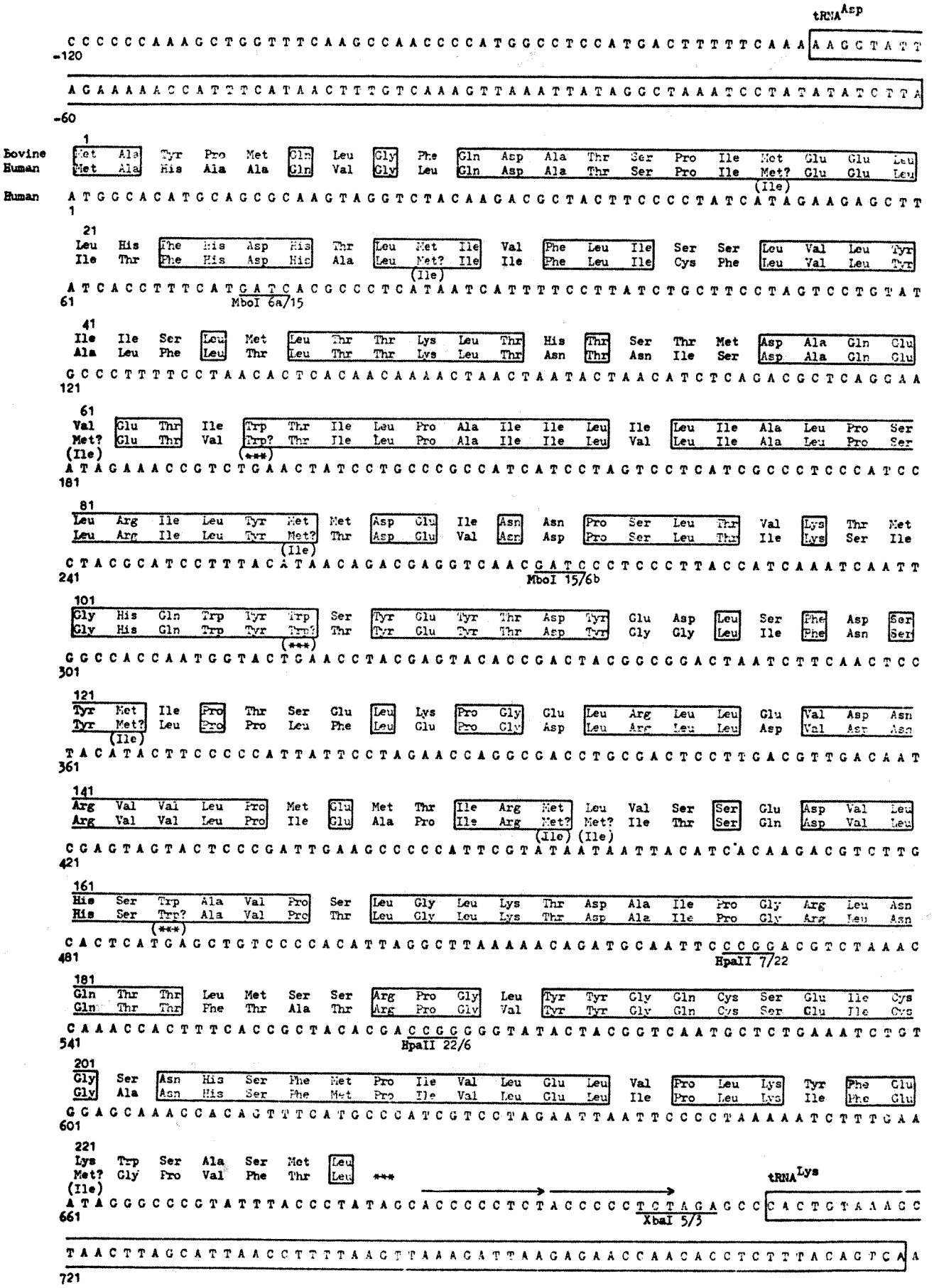


Fig. 2 The sequence of the COII gene region showing the position of the two tRNA genes and the COII DNA sequence aligned with the bovine COII protein sequence[1]. The predicted human protein sequence is compared with the bovine sequence and homologous amino acids are shown boxed using the codons AUA as methionine and UGA as tryptophan. Their normal assignments isoleucine and termination (denoted by ***) are shown underneath. The sequence can be aligned with the genetic and restriction maps shown in Fig. 1 by the restriction enzyme sites *Mbo*I and *Xba*I shown. A 9-base repeat at the end of the COII gene is also shown.

the beginning of the COII gene to the *Mbo*I site at position +73, and Fig. 3*b* shows the sequence obtained using *Hpa*II fragment 22 as a primer from position +521 to the *Mbo*I site at position +274. The positions of the TGA and ATA codons in these regions are shown.

tRNA genes

Three tRNA genes have been mapped in this region of the genome[2], two hybridising to the H strand and one to the L strand (see Fig. 1). In the sequence presented in Fig. 2 we have only been able to locate two tRNA genes, both of which are coded on the L strand, that is, the tRNA products would hybridise to the H strand. The locations of these tRNAs agree well with the hybridisation map positions[2] shown in Fig. 1, and from their positions in the sequence they probably correspond to H5 and H6 according to the nomenclature of Angerer *et al.*[2]. However, if this assignment is correct there should be a tRNA gene close to the $tRNA^{Lys}$ gene on the opposite strand that we have been unable to find. The tRNA genes were located by searching for base-paired cloverleaf structures using the computer programs HAIRPN[13] and TRNA (R. Staden, unpublished).

The sequences of the two putative tRNA genes are shown in the cloverleaf form in Fig. 4. If these tRNAs have CCA at their 3′ ends then this is not encoded in the DNA. The sequences in the D loop (left-hand loop) and the TψC loop (right-hand loop) of both tRNA genes differ considerably from the corresponding sequences found in all other tRNAs (see collection of 209 tRNA sequences compiled by Sprinzl *et al.*[14]). In the tertiary structure of yeast $tRNA^{Phe}$ (refs 15, 16) these two loops are hydrogen bonded together by conserved nucleotides not found in the two mitochondrial tRNA sequences. An examination of the primary structures of the two genes in these regions has not yet revealed how their respective D and TψC loops interact in the manner of yeast $tRNA^{Phe}$. A more detailed discussion of these and other human mitochondrial tRNA genes will be published separately.

UGA and UGG are both tryptophan codons in human mtDNA

The most interesting aspect of the sequence is in the part coding for the COII gene which is identified by the high homology with the bovine protein sequence[1]. When the human DNA sequence is aligned with the beef protein sequence as shown in Fig. 2, it becomes apparent that there are three 'in phase' UGA (*opal*) termination codons within the gene. The possibility that these are present in intervening sequences can be excluded by several criteria. First, the gene seems to be the exact length needed to code for its product, without interruption, from the AUG initiation codon to the UAG (*amber*) termination codon. Second, the UGA codons are in regions of very high homology between the beef and predicted human protein sequences. Finally, and most important, is the fact that they all align with tryptophans in the bovine protein. This suggests very strongly that UGA is a tryptophan codon in this genetic system and not a termination codon. There are five tryptophan residues in the bovine COII sequence, three of them opposite UGA codons, one opposite a UGG tryptophan codon and one opposite a GGG glycine codon. The latter is in a region where there is no homology with the bovine protein sequence at position 664 in the DNA sequence shown in Fig. 2. Tryptophan is normally coded for by a single codon, UGG, which is translated by a $tRNA^{Trp}$ with a CCA anticodon in both prokaryotes[17] and eukaryotes[18,19]. However, there is a precedent for UGA being used as a tryptophan codon. $tRNA^{Trp}$ from an *su*$^+$ UGA strain of *E. coli* (CAJ64) suppresses a UGA mutation in lysozyme mRNA during cell-free protein synthesis and synthesises polytryptophan from a repeating UGA polymer[20,21]. However, in both assays there was a low synthesis of product using $tRNA^{Trp}$ from the *su*$^-$ *E. coli* CA244. This, coupled with the observation that *in vivo* UGA codons are leaky in *su*$^-$ strains[22,23], suggested that the low level of UGA suppression in *su*$^-$ strains was caused by the normal $tRNA^{Trp}$. Also the low level of readthrough of the Qβ coat protein UGA termination codon in *su*$^-$ hosts was shown to occur by insertion of tryptophan and a functional longer protein necessary for infectivity was found in the phage particle[24,25]. Thus, there is a precedent in prokaryotes that UGA can be used as a tryptophan codon, albeit infrequently.

The codons UGA and UGG are adjacent in the genetic code (see Fig. 5) and could thus be recognised specifically by a $tRNA^{Trp}$ having the anticodon UCA according to the 'wobble hypothesis' (ref. 26) where U in the first position of the anticodon could pair with A or G in the third position of the codon. However, $tRNA^{Trp}$ from both the *su*$^-$ and *su*$^+$ UGA *E. coli* strains have CCA as their anticodons and the suppressor $tRNA^{Trp}$ has a G24 → A24 in the semi-invariant base pair in the 'D arm' (ref. 17). This must somehow alter the tertiary structure of the suppressor tRNA to allow the C in the first position of the anticodon to pair with the A in the third position of the UGA codon. Although neither the human mitochondrial $tRNA^{Trp}$ nor its gene has yet been characterised there is evidence from charging experiments[4] of unfractionated mitochondrial tRNA for a mitochondrially coded $tRNA^{Trp}$.

It thus seems clear that if this gene is expressed in its entirety, UGA and UGG are both used as tryptophan codons in the genetic code of human mitochondrial DNA. The sequence of the yeast mitochondrial COII gene also has UGA codons opposite tryptophan residues when it is aligned with the bovine COII protein sequence[27,41]. The use of UGA as a tryptophan codon would preclude the use of the cytoplasmic termination factor which corresponds to all three termination codons[28]. Prokaryotes use two factors, R1, which corresponds to UAA and UAG, and R2, which corresponds to UAA and UGA[29]. Thus, it is likely that the mitochondria has a factor analogous to R1.

Does AUA code for methionine in human mtDNA?

There are two codons in the genetic code that are each the sole codon for a single amino acid. The case of UGG for tryptophan has been discussed above. The other codon is AUG, which codes for methionine, and in the same box isoleucine is encoded by AUC, AUU and AUA. All other boxes can be read using G–U wobble[25] by one tRNA that is specific for codons ending in C and U and another that is specific for codons ending in A and G. The main exception to this is when the anticodon contains modified bases that can restrict the coding properties. Thus, with the UGA/tryptophan precedent in mind, can AUA code for methionine? There are 15 internal methionine residues in the beef COII sequence and only one of these is aligned with an AUG codon but five of them are aligned with AUA codons (Fig. 2). There are eight AUA codons, five aligned with methionine, one with valine, one with leucine and one with lysine. None is aligned with isoleucine codons. Thus, there are two possibilities: either by coincidence the bovine methionine residues in these positions are being preferentially replaced by isoleucines which are all coded for by AUA, or there is another change in the code and AUA is being recognised as a methionine codon. AUA and AUG could both be recognised by a tRNA having the anticodon UAU according to the wobble hypothesis[26].

In view of the UGA/UGG precedent we think that the above data suggest that AUA and AUG are methionine codons. However, isoleucine and methionine are both hydrophobic amino acids and confirmation will depend on the determination of the human COII protein sequence.

If it is assumed that AUA is a methionine codon and UGA a tryptophan codon, the bovine protein and the predicted human protein sequences are 72% identical. The conserved amino acids are shown boxed in Fig. 2. This leaves 62 out of 227 amino acids in bovine COII that are different in the human COII. It is striking that 22 of these differences involve either methionine or serine aligning with other amino acids, eight of them threonine, using the codons ACA and ACC. However, both of these codons also align with threonine, and with the data shown in

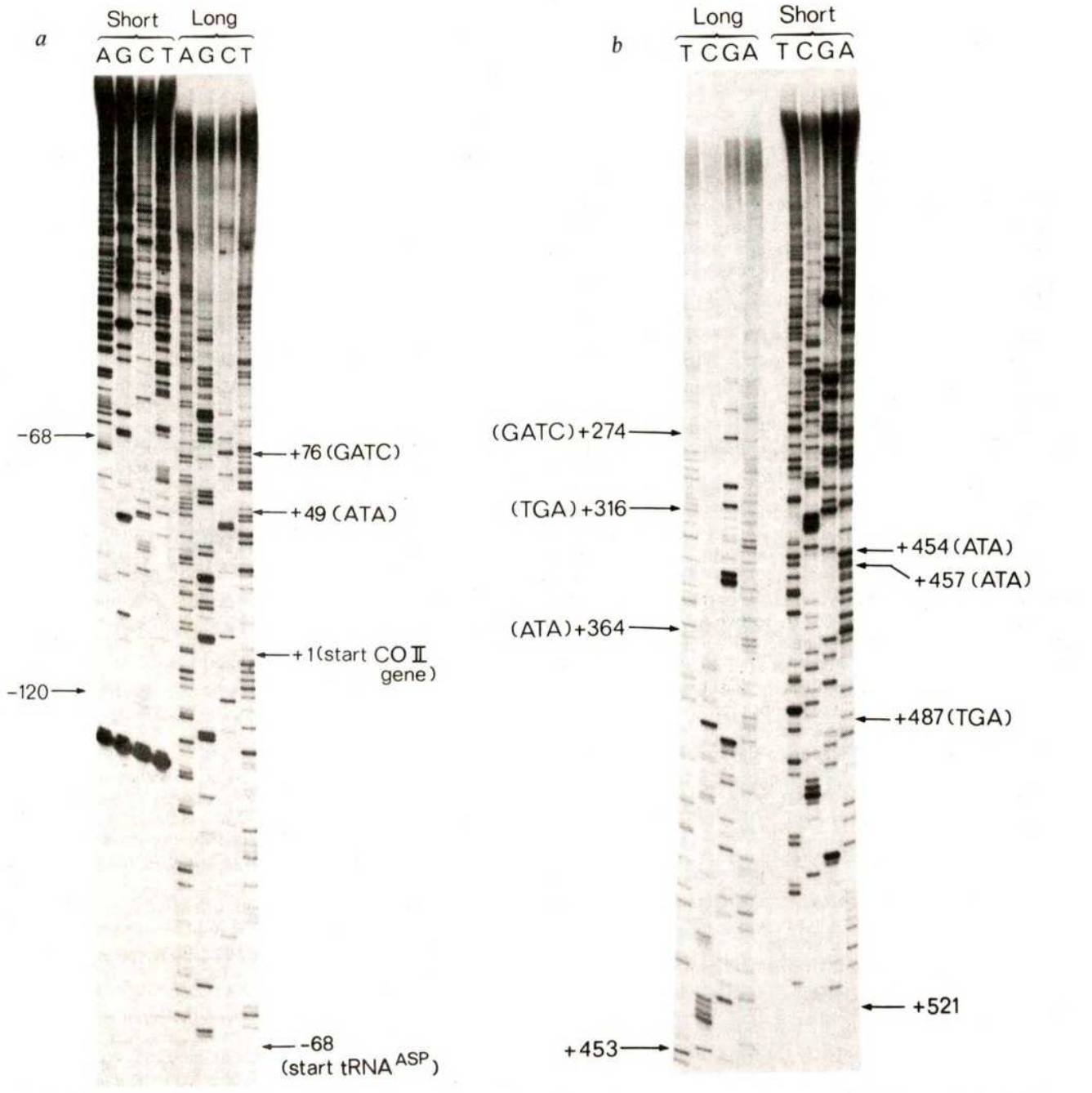


Fig. 3 Two examples of chain termination sequencing gels in the COII gene region. *a* Shows the use of a small restriction fragment primer in *Mbo*I fragment 6a primed on 'H-strand' template prepared from clone pmt 110 (see text) by the exonuclease III method of Smith[12]. The sequence corresponding to that given in Fig. 2 can be read from base −120 to the *Mbo*I site at +76 which is the junction between the clone of *Mbo*I 6a and pBR322. The starts of the $tRNA^{Asp}$ gene and the COII gene are shown along with the ATA codon at position +49. A short run and a long run of the sequencing reactions on the same 8% polyacrylamide thin gel[46] are shown. *b* Shows a similar experiment using the fragment *Hpa*II 22 as a primer on 'L-strand' template prepared from clone pmt 110 (*Mbo*I fragment 6b, see text). For ease of reading the track order A, G, C, T has been changed to the complementary order T, C, G, A so that the gel can be read as the L-strand sequence 5′ to 3′, from top to bottom. The sequence from the junction between pBR322 and *Mbo*I 6b at position +274 can be read to position +521. The positions of the UGA and AUA codons are shown.

Fig. 2 there is no clear indication of other coding changes. The one difference between the yeast mitochondrial ATPase subunit 9 protein sequence[30] and the sequence of its gene[31] is a threonine aligned with a CUA codon. In the human COII gene, 11 out of 12 CUA codons align with leucine so that this cannot be a coding change in human mtDNA. However, it is possible that other coding changes will be found in mtDNA and there may be different codes in the mitochondria of different species.

Codon usage in the COII gene

Figure 5 shows the suggested code in human mitochondria with AUA as possibly methionine and UGA as tryptophan. The number of times each codon appears in the COII gene is also shown. All amino acids are used and nearly all codons. Only one of the serine AGU/C set is used and none of the arginine AGA/G set. Excluding the UAG termination codon, only 16 out of 227 codons end in G. This reflects the low G content found in the L strand generally (unpublished results). Specifically in this region the composition is A, 29.9%; C, 30.2%; T, 25.8% and G, 14.1%. The low G content may be of significance when considering the evolution and use of the coding changes proposed here. Including AUA as methionine and UGA as tryptophan the ratio of codons ending in A to those ending in G is 5:1.

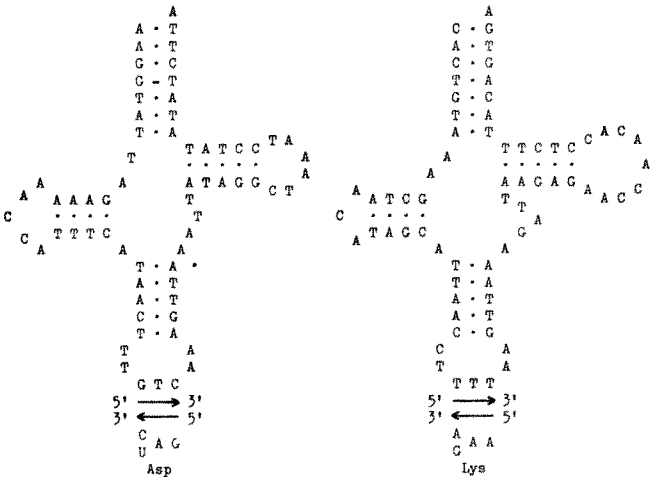


Fig. 4 The DNA sequences of the $tRNA^{Asp}$ and $rRNA^{Lys}$ genes are shown arranged in the cloverleaf conformation. The CCA sequence found at the 3′ end of all tRNAs is not encoded in the DNA sequence. The presumed codons that these tRNAs would recognise by G–U wobble[26] are indicated.

It is also interesting to consider the codon usage in relation to the number of tRNAs found to be encoded by human mtDNA. The highest number of tRNAs so far found to be coded by mammalian mtDNA is 21 (ref. 32) out of the minimum number required using G–U wobble of 32. If the AUA coding change is substantiated then a minimum of 31 would be required. On the basis of the relatively small sample of codons of the COII gene, it is probably reasonable to assume that in the whole genome all codons are used, although some may be used rarely. There is no evidence in mammalian mitochondria to suggest that any RNA is imported, and so there may be a complete set of mitochondrially coded tRNAs. Otherwise, it is difficult to see how 21 tRNAs could recognise all codons without some degree of misreading. However, it is possible that the smaller rRNAs, the novel features of the tRNAs, the possible coding changes discussed here and a limited set of tRNAs might indicate that a different protein synthesis decoding mechanism operates in the mitochondria. Selective use of a 'two out of three mechanism' (refs 33, 34) would only require 23 or 24 tRNAs, depending on the coding changes.

Organisation of genes in the COII region

Attardi *et al.*[7] discussed two models for the organisation of the tRNA genes with respect to the protein coding sequences. As can be seen in Fig. 1 the tRNA genes are scattered more or less uniformly around the genome and on both strands. These genes could either separate the sequences coding for the mRNA (the punctuation model) or could reside in them in the manner of intervening sequences (the insert model). The COII gene is flanked by two tRNA genes as shown in Fig. 2 but surprisingly, the $tRNA^{Asp}$ gene is actually contiguous with the COII gene, with no noncoding nucleotides in between. Thus, it is possible that if there are sequences involved in ribosome recognition preceding the initiation codon these lie in the $tRNA^{Asp}$ gene. No sequence corresponding to the *E. coli* ribosome recognition site is found[35,36]. There is a sequence similar to that postulated to have the same function in eukaryotes[37] but there is no evidence that this interaction occurs in eukaryotes and in any case mitochondria have their own rRNAs, which are much smaller than both their prokaryote and eukaryote counterparts. The possibility of a leader peptide which is subsequently processed is ruled out by in-phase UAA termination codons preceding the initiation codon.

The contiguous nature of the two genes raises the obvious difficulty in processing the primary transcript. Both strands of the mtDNA have been shown to be completely transcribed and it is not known whether there are additional promoters. If a full length transcript of the H strand is processed to give the rRNAs, and the tRNAs and mRNAs, the latter being polyadenylated at some stage, then a single cleavage between the two genes would leave no 5′-leader sequence on the COII gene mRNA. Therefore, if initiation of translation of the COII mRNA required signals in the sequence preceding the initiation codon, the tRNA would either have to be released after the cessation of translation of the mRNA or have to be transcribed separately. An intriguing possibility is that the tRNA could be spliced out of the transcript in the manner of an intervening sequence, although this seems unlikely; no evidence has yet been found for intervening sequences in human mtDNA.

There is a short noncoding region (25 bases) between the end of the COII gene and the $tRNA^{Lys}$ gene and this contains a 9-base repeat, as shown in Fig. 2. The sequence AAUAAA is presumed to be involved in polyadenylation of eukaryotic mRNAs and the nearest is 224 bases downstream of the 3′ end of the COII gene (unpublished results). If this is the signal for polyadenylation of the COII mRNA, a similar problem to that of the $tRNA^{Asp}$ regarding the processing of the $tRNA^{Lys}$ arises.

There has been considerable speculation that mitochondria have evolved from bacteria that had entered into a symbiotic relationship with their host (for review see ref. 6). The economical use of the coding capacity of the DNA is more characteristic of prokaryotes, in particular bacteriophage DNAs such as ΦX (ref. 38), but the eukaryotic viruses such as SV40 (refs 39, 40) show similar economy. Thus, it is difficult to draw any conclusions as to whether this genetic system is more similar to prokaryotes or eukaryotes on this basis. If the endosymbiotic theory is correct, the different genetic code may represent a 'frozen', more primitive code, for it is unlikely that once mitochondria became essential to their 'host' the code would be able to change. Whatever their origins the novel features of the mitochondria discussed here seem to require that they be considered a separate class from the prokaryotic and eukaryotic genetic systems.

Since this paper was submitted, Macino *et al.*[41] have also demonstrated the use of UGA as tryptophan in the yeast mtDNA COII gene. Also, recent work by F. Sanger and A. R. Coulson (personal communication) has identified the sequence coding for the tryptophan tRNA gene in human mtDNA. As predicted, this structure has the anticodon UCA which would pair with UGA and UGG using G–U wobble.

We thank Dr G. Buse for communicating to us the bovine COII protein sequence before publication, and Drs F. Sanger and J. E. Walker for advice and helpful discussions.

First letter	Second letter: U			C			A			G			Third letter
U	UUU	Phe	3	UCU	Ser	1	UAU	Tyr	1	UGU	Cys	1	U
	UUC		7	UCC		4	UAC		8	UGC		2	C
	UUA	Leu	4	UCA		4	UAA	Ter	0	†UGA	Trp	3	A
	UUG		1	UCG		0	UAG		1*	UGG		1	G
C	CUU	Leu	7	CCU	Pro	1	CAU	His	2	CGU	Arg	2	U
	CUC		6	CCC		9	CAC		4	CGC		1	C
	CUA		12	CCA		3	CAA	Gln	6	CGA		3	A
	CUG		3	CCG		2	CAG		1	CGG		0	G
A	AUU	Ile	7	ACU	Thr	5	AAU	Asn	2	AGU	Ser	1	U
	AUC		15	ACC		8	AAC		5	AGC		0	C
	†AUA	Met	8	ACA		8	AAA	Lys	4	AGA	Arg	0	A
	AUG		2	ACG		0	AAG		0	AGG		0	G
G	GUU	Val	1	GCU	Ala	4	GAU	Asp	3	GGU	Gly	2	U
	GUC		7	GCC		5	GAC		8	GGC		4	C
	GUA		5	GCA		4	GAA	Glu	8	GGA		3	A
	GUG		0	GCG		1	GAG		3	GGG		2	G

Fig. 5 The code in human mtDNA suggested by the results presented here is shown along with the number of times each codon is used in the COII gene†AUA and UGA are normally isoleucine and termination, respectively.

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letters

Where can antimatter be hidden in the Universe?

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Undoubtedly complete symmetry of charge in the initial state of the Universe is an attractive idea. However, analysis of observational data[1] indicates that the visible Universe contains mostly particles of positive baryon number. Attempts[2–6] to reconcile the assumption of initial symmetry with this observed asymmetry have met with some difficulties. However, if one accepts that the Universe is symmetric in baryon charge, then there must be regions of space or objects which contain at least as much antimatter as the matter which we observe. We propose here that the hypothesis of primordial black holes (PBHs)[7,8] offers a simple and economic solution of the problem. Because of the well-known 'no hair' theorems the baryonic charge of PBHs cannot be observed, and hence PBHs are ideal stores for antimatter.

Extra antibaryons can be captured by PBHs either because of fluctuations in the density of the baryonic charge[9] or for dynamic reasons. Here we concentrate on the first possibility: we assume that in the early Universe the PBH happened to collect excess antibaryons. Then, after baryon–antibaryon annihilation we arrive at the present charge asymmetry.

To obtain some quantitative estimates we assume that in the hadronic era M black holes were created each containing N_i^+ baryons and N_i^- antibaryons and that their distribution in the ith PBH ($i = 1, 2, \ldots, M$) is the gaussian

$$W(N_i^{\pm}) = (2\pi\overline{\Delta N_i^2})^{-1/2} \exp\{-(N_i^{\pm} - \bar{N}_i)^2/2\overline{\Delta N_i^2}\} \quad (1)$$

The mean number of particles $\bar{N}_i$ and the fluctuations $\overline{\Delta N_i^2}$ are taken to be equal for particles and antiparticles because of the charge symmetry of the strong interaction. The probability that the PBH contains N_u extra antibaryons is

$$W(N_u) = \int_0^{\infty} \left\{ \prod^{M} \frac{dN_i^+ \, dN_i^-}{2\pi\overline{\Delta N_i^2}} \exp\left[-\frac{(N_i^+ - \bar{N}_i)^2 + (N_i^- - \bar{N}_i)^2}{2\overline{\Delta N_i^2}} \right] \right\}$$

$$\times \delta\left[\sum_{i=1}^{M} (N_i^- - N_i^+) - N_u \right] \quad (2)$$

$$= \left(4\pi \sum_{i=1}^{M} \overline{\Delta N_i^2} \right)^{-1/2} \exp\left(-\frac{N_u^2}{4\sum_{i=1}^{M} \overline{\Delta N_i^2}} \right)$$

Equation (2) shows that the most favourable situation corresponds to a minimum (mean) asymmetry of black holes: the greater the mass contained in a PBH (the more it increases $\sum^M \overline{\Delta N_i^2}$), the larger is the deviation.

Let us now estimate the maximum energy density (averaged over the whole of space) of unobservable forms of matter assuming the present value of the Hubble constant H and that the age of the Universe ($\sim 2 \times 10^{10}$ Myr) exceeds the age of the Earth ($\sim 5 \times 10^{9}$ Myr). This gives

$$Ht \leq f(\Omega)$$

where $\Omega = \rho/\rho_c$ is the ratio of the energy density of the whole matter to the critical density, and $f(\Omega)$ for the closed Universe has the form

$$f(\Omega) = \frac{\Omega}{(\Omega-1)^{3/2}} \left[\arcsin\left(\frac{\Omega-1}{\Omega}\right)^{1/2} - \left(\frac{\Omega-1}{\Omega}\right)^{1/2} \right]$$

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It follows that

$$\Omega \leqslant 62.5$$

As the density of observable forms of matter $\rho \approx 0.03\rho_c$, then the ratio of the energy density of unobservable to observable forms of matter can reach 2×10^3 and, consequently, the mean asymmetry in PBH is $\sim 5\times10^{-4}$.

If matter in its early stages can be considered as an ideal gas, then $\Delta N_i^2 \sim N_i$ and the probability $W(N_u)$ for N_u baryons in the Universe will be

$$W(N_u) \sim \exp\left\{-\frac{N_u^2}{N}\right\} = \exp\{-\sigma N_u\}$$

where σ is a measure of the asymmetry in PBHs. As should have been expected, an asymmetric creation of PBHs is highly unlikely. Nevertheless 'the dice were cast only once', which gives us the opportunity to analyse the evolution of the Universe.

Note that the possibility for an asymmetric creation of PBHs can be considerably increased if matter at the early stage satisfies a non-ideal equation of state. For example, for van der Waals equation the free energy has the form[10]

$$F = -NT\ln\frac{eVn_0(T)}{N} - NT\ln\left(1-\frac{Nb}{V}\right) + a^2\frac{N^2}{V}$$

and

$$\overline{\Delta N^2} = T\left(\frac{\partial N}{\partial \mu}\right)_{V,T} = T\left(\frac{\partial^2 F}{\partial N^2}\right)^{-1}_{V,T} = \left[\frac{V^2}{N(V-Nb)^2} - \frac{2a}{TV}\right]^{-1}$$

Hence in the vicinity of a phase transition, $\overline{\Delta N^2}$ may become arbitrarily large and make the capture of extra antibaryons in PBHs probable. A van der Waals system of ultrarelativistic particles has been considered in detail elsewhere[11].

Note also, that this probability makes sense either when an ensemble of systems is indicated or when the event is repeated in time. Therefore, for the Universe as a whole it should be used with care. Of course, one can assume (without any hope of confirmation) that there are many universes which like ours originated in a big bangs; we then live in 'the best of all worlds': in the world in which a fraction of the antimatter has been hidden in PBHs. Another possibility concerns an oscillating Universe, in which expansions are followed by collapses. In this case we should have to wait for the situation described above to be realised (with the formation of asymmetric PBHs).

The account of the evaporation of black holes given by Hawking[12] does not essentially change the situation; it only imposes limitations on the mass spectrum of PBHs[13]. Moreover, as the evaporation of black holes is a charge-symmetric process, the extra baryonic charge captured by PBHs will not be observable, even if the evaporation ceased and created a naked singularity, the maximon[14].

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Structure of the uranian upper atmosphere

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Although the occultations of relatively bright stars by planets are rare events, they provide an important ground-based method for determining temperature, pressure and number density profiles of planetary upper atmospheres[1]. The technique is particularly valuable for planets in the outer Solar System, whose atmospheres have not yet been probed by spacecraft missions. Since the atmosphere of Uranus had not yet been investigated by the occultation technique, we have observed and report here the occultation of the star SAO158687 by Uranus on 10 March 1977 (refs 2, 3).

A photometer was set up on the 0.46-m reflector at the Cape Observatory, South Africa that used an RCA photomultiplier with a gallium arsenide photocathode, and a 3-mm thick Schott RGN9 filter. The data were recorded on one channel of a chart recorder, while radio time signals were recorded on another channel. The time constant of the data recording system was ~3 s and the signal from the star produced a deflection of 1.0 cm on the chart. The chart was measured at intervals of 0.5 s.

The light curves were analysed using the inversion method of French, Elliot and Gierasch[4]. This procedure gives the number density, pressure and temperature of the occulting atmosphere as well as the uncertainties in these values due to shot noise in the light curve. The derived temperature profiles for immersion and emersion are plotted as a function of number density in Fig. 1. The shaded upper section represents an isothermal atmosphere fit to the initial part of the light curve, which provides definite initial conditions with which to begin the numerical inversion calculation. The inversion was terminated when the uncertainty in the baseline of the light curve produced an error in the temperature equal to the shot noise error. The error bars in Fig. 1 have a total length of two standard deviations, and represent the uncertainty in the overall placement of the profile at that point. The small scale structure is more reliable than the overall placement of the curve because the error in the placement of successive points is strongly correlated[4,5].

The fundamental quantity obtained from the inversion is the scale height H which is related to the temperature T of the atmosphere, through the equation $T = \mu gH/R$, where μ is the mean molecular weight of the atmosphere, g is the local gravitational acceleration and R is the universal gas constant. To establish the temperature scales in Fig. 1 we assumed $g = 830\ \mathrm{cm\ s^{-2}}$ and $\mu = 2.2\ \mathrm{g\ mol^{-1}}$, which corresponds to an atmosphere of H_2 and He in their solar abundances. If this assumption about the composition is wrong, the scale heights in Fig. 1 will not be affected, but the temperature scales will have an error proportional to the ratio of the true mean molecular weight to that assumed here.

The mean temperature of the inverted section of the profile is 87 K for immersion and 90 K for emersion. The temperature derived by fitting an isothermal atmosphere occultation light curve[6] to the data is 104 K for immersion and 108 K for emersion. Because the atmosphere is strongly non-isothermal, we feel that the mean inversion temperature should be a more reliable indicator of the mean atmospheric temperature than the isothermal fit temperature.

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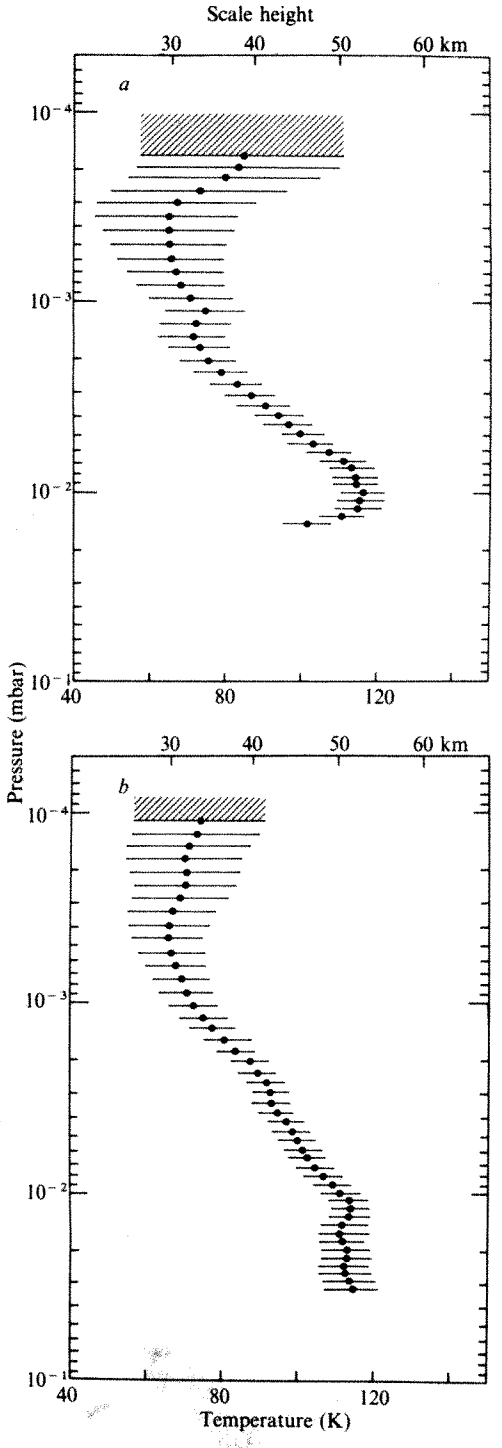


Fig. 1 Temperature profiles of the uranian upper atmosphere for: *a*, 7° N lat, Cape Town immersion; and, *b*, 21° S lat, Cape Town emersion. The shaded areas are the regions of the isothermal fit boundary condition. The level at half light is indicated on the right, while the temperature from a full isothermal fit is indicated at the bottom. Note the large variations in temperature.

At the time of the occultation, the subsolar point on Uranus was located at latitude +51.0° and the phase angle was 2.4°. The immersion and emersion points as seen from the Cape were at latitude 7.3° N and 20.9° S respectively, with immersion occurring just before sunrise and emersion just after sunrise. Our results indicate no large difference in the mean temperature for these two conditions.

Both temperature profiles show large variations, ~50 K peak-to-peak for immersion and 40 K for emersion, with a vertical length scale of ~150 km. Although some of this variation is probably due to noise, we believe that most of these variations represent real temperature variations in the atmosphere of Uranus. First, they are much larger than any variations one might expect from shot noise in the light curve[4] and second, the profiles agree well with the Kuiper Airborne Observatory (KAO) results[7,8]. The regions of the uranian atmosphere probed by observations from the Cape and the KAO correspond to the same levels in the atmosphere, but are displaced from each other about 2,200 km along the limb of Uranus for the immersion data and 2,600 km for the emersion data.

The mean atmospheric temperature found here (~90 K) is somewhat cooler than the 140 K found for the upper atmosphere of Neptune from a previous stellar occultation[9]. It is also cooler than that obtained by Wallace[10] from model calculations. Further work will be required to find the reasons for these differences and to establish the cause of the observed 'wavelike' temperature variations. The main problem with dynamical explanations for the wavelike structures is that the obvious wave mechanisms—vertically propagating density waves, for example—apparently heat the atmosphere to much higher mean temperatures than we observed[8]. A more likely possibility is that the variations are caused by photochemical processes[8].

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Multiply-twinned particles in silver catalysts

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It has been known for several years that anomalous structures can occur in the early stages of particulate growth. Ino[1] and Ino and Ogawa[2] for gold on rocksalt and Allpress and Sanders[3] for nickel on mica found small polycrystalline nuclei which they interpreted as multiply-twinned particles (MTPs). These particles can be considered as 5 or 20 f.c.c. tetrahedra joined by twin boundaries to give decahedra or icosahedra (Fig. 1). As the tetrahedra do not assemble to a completely space filling structure, either elastic strains or grain boundaries are required to complete the particles. More recently, MTPs have been observed in the early stages of growth for all f.c.c. metals in certain conditions. Gillet[4] has recently reviewed the field whilst Hayashi *et al.*[5] give extensive results for f.c.c. metals in argon smokes. The possible significance of MTPs in heterogeneous catalysts was first suggested by Allpress and Sanders[6] and investigated by Avery and Sanders[7]. They found, however, that at most 2% of particles 100–350 Å in size for Ni, Au, Pt and Pd on γ-alumina were multiply twinned. We report here the presence of considerable numbers of multiply-twinned particles in various heterogeneous metal catalysts, in particular silver on α-alumina. The particles can have quite large dimensions up to ~2,000 Å. Attention is drawn to the possible significance of some special sites on these particles which have no counterpart on single crystal surfaces, and to the unusual distribution of surface faces.

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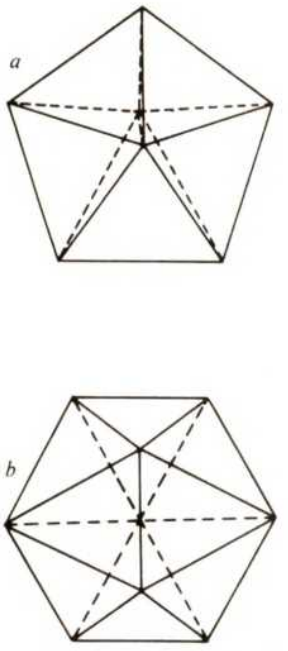


Fig. 1 Structure of *a*, decahedral and *b*, icosahedral multiply-twinned particles.

The samples examined were supplied by ICI Ltd, having been prepared by the following method (UK Patent 1369639): an $0.8\,M^2g^{-1}$ support of α-alumina (Carborundum Ltd, No. SAHT96) was immersed in a solution of silver oxalate in 45% water—45% ethylene diamine—10% ethanolamine. The support was then pyrolysed in nitrogen at 200 °C for 1 h to decompose the oxalate to silver metal. The catalyst examined contained approximately 16% silver by weight. Samples were prepared for examination in a Siemens 102 microscope by allowing a drop of ground catalyst suspended in acetone to dry on amorphous carbon films premounted on microscope grids.

The samples examined contained principally isolated particles of size between 1,000 and 2,000 Å, although smaller particles (<500 Å) were found associated with amorphous regions of the substrate, and a few large polycrystals were observed, presumably due to particle coalescence. The discrete particles displayed well developed 111 facets, with very little edge or corner rounding. More than 80% of the discrete particles were observed to be MTPs, the remainder being composed mainly of twinned platelets, faulted needles and single crystals. Micrographs of these various morphologies are shown in Figs 2 and 3. All the icosahedral MTPs and most (but not all) of the decahedral MTPs displayed internal contrast suggestive of dislocations, as has been previously noted for large MTPs[8].

The occurrence of MTPs in heterogeneous catalysts is consistent with their common observation in epitaxial films. MTPs may well occur in many other catalysts; we have, for example, examined micrographs of a nickel catalyst (A. Szczepura, personal communication) which show contrast typical of MTPs. The apparent contradiction between our results and those of Avery and Sanders[7] is probably due to the different methods of production; the particles described here were prepared by a mild pyrolysis, whereas Avery and Sanders examined their particles after annealing in hydrogen. Preliminary results for <50 Å gold particles on NaCl or amorphous Al_2O_3 have shown that the particles are single crystals after annealing at 500 °C in hydrogen but multiply twinned after annealing at 500 °C in air. It seems probable that the occurrence and survival of MTPs in catalysts may depend on the atmosphere present during both preparation and running.

The large sizes of the MTPs observed is probably a result of the growth conditions. As the type of particle observed corresponds with the types commonly found for small (<200 Å) metal particles, it seems likely that the internal structure was determined at some early stage in the growth. Further layer growth, possibly autocatalytic in nature, could produce the observed particles. In the absence of sufficient activation energy from processes such as coalescence, the particles can remain in the multiply twinned structure, even though this is almost certainly metastable in this size range. Given the existence of the multiply twinned structure, the equilibrium of the surface faceting is an additional question. More detailed experimental and theoretical studies of this problem, which will be published elsewhere, suggest that the form observed here, with sharp 111 facets, may in fact be metastable with respect to surface changes at room temperatures and that the stable structure would have additional 100 facets as well as notches at the boundaries.

One interesting feature of the MTP is that they have some unusual surface sites, some of which have no analogue on a single crystal surface. On the actual twin boundary the atoms have h.c.p. coordination rather than f.c.c. so that the *d*-bonds will be differently orientated. Also, as mentioned above, more complicated faceting geometries including notches may arise if the surfaces attain their equilibrium configuration. Such notches or re-entrant surfaces have been considered to act as preferential nucleation sites for crystal growth[9] and may have some catalytic importance. For a 50-Å radius particle approximately 7% of the surface atoms would be on the twin boundaries, a third of these at the notches.

Anomalous surface sites associated with twins are, of course, also found in other particles. Re-entrant surfaces have been observed for f.c.c. particles with an odd number of closely spaced parallel twin boundaries by Hayashi *et al.*[5]. However, the twin density in MTPs will usually be higher than in other particles.

The fact that MTP surfaces will usually consist of a different distribution of crystal faces than would occur in a single crystal could also be of significance in catalytic reactions which are sensitive to surface crystallography. It seems likely that MTPs will have an anomalously high fraction of their surface as (111) planes and, in addition, the icosahedra will not have any (100) surface planes.

Because the tetrahedral units are not completely space-filling the MTPs probably contain considerable elastic strain, or possibly crystal defects such as dislocations as observed in Si particles by Saito[8]. In either case the catalytic properties may be

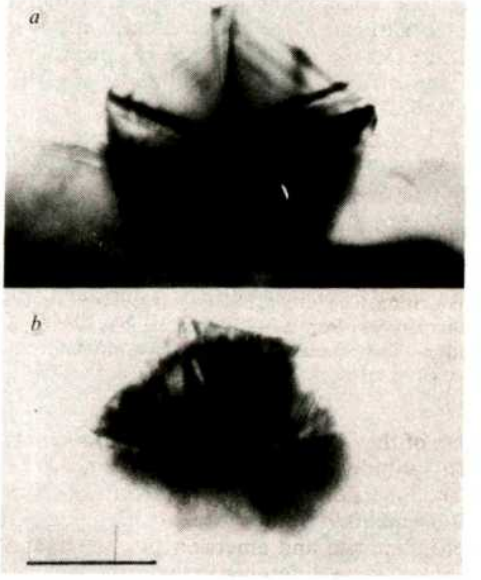


Fig. 2 Micrographs of *a*, Decahedral MTP near the 5-fold symmetry axis and *b*, icosahedral MTP near the 'face' orientation. Scale bar, 0.1 μm.

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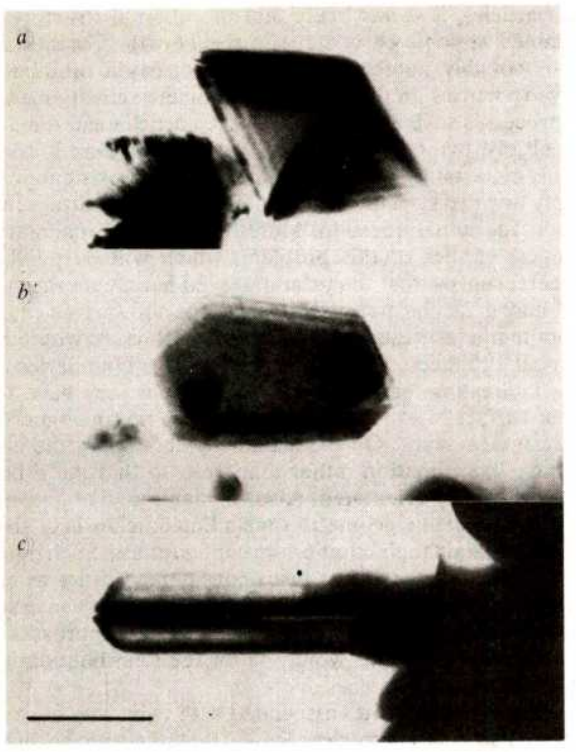


Fig. 3 Micrographs of *a*, single crystal (square pyramid); *b*, platelet and *c*, needle. Scale bar, 0.1 μm.

affected. Ino[10] assumed a homogeneous elastic strain maintained by external forces to estimate the elastic strain energy using anisotropic elasticity theory. His figures for Ag indicate that the lattice parameters in the surface of the icosahedron are expanded by about 3%, while the spacing of planes parallel to the surface is reduced by nearly 5%. The lattice parameter in the surface of the decahedron is increased by 1% in the direction of the ⟨110⟩ outer perimeter and reduced by 1% in the direction normal to this. Removal of Ino's external forces will lead to additional inhomogeneous elastic strains. De Wit[11], for instance, has pointed out the similarity between the decahedron and a disclination which has very high compressions close to the central axis. In the icosahedron the stress is probably also inhomogeneous, being highly compressive at the centre and tensile at the surface. More detailed calculations have been made but these are complicated by the uncertain effects of surface tension stresses in such small particles (A.H., L.D.M. and E. H. Yoffe, in preparation).

Clearly it would be of considerable interest to produce catalyst samples containing, for example, a larger proportion of single crystal particles in the same size range as the MTPs. It might then be possible to make a direct experimental assessment of the practical significance of these anomalous structures in catalysis.

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Propagating chemical waves in the uncatalysed gallic acid–bromate system

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Chemical oscillatory systems which include at least one autocatalytic reaction step are of interest as thermodynamically open systems in which temporal and spatial structures are produced. These systems also show other types of unusual kinetic behaviour[1]. To analyse the elementary steps of their kinetics the simplest possible reactions are needed especially in relation to nonlinear thermodynamics[2]. The theory of these chemical reactions is still only at the level of a simplified mathematical model, and theoretical results are in poor agreement with experimental findings. Therefore it is important to study other examples of spatial dissipative structures corresponding to chemical systems which exhibit oscillations. We report here observations of the oscillating reaction between bromate and the phenolic compound gallic acid in which propagating chemical waves occur.

The Belousov–Zhabotinskii reaction system (BZR) is known to exhibit propagating chemical waves[3]. This reaction is very complex and involves about 20 elementary steps (the mechanism has been elucidated by Field *et al.*[4]) starting from an initially homogenous state. Until now no other chemical reaction starting from this condition has been found to form a spatial structure spontaneously (unlike a Liesegang ring-type one).

The BZR requires a transition metal catalyst but the rate law of wave propagation shows the propagation velocity to be independent of the concentration of the catalyst in the range studied by Noyes and Field[5]. This means that the step catalysed by the transition metal ion, although necessary for oscillation and/or wave propagation in the BZR does not determine the propagation velocity.

Körös and Orban[6] have reported a new oscillatory chemical reaction between bromate and phenolic or aniline-type compounds, occurring in the absence of a catalyst. (A catalyst may be added, but the reaction then becomes of the normal BZR type[7,8].) A similar reaction between bromate, and bisazonium salts was reported previously by Kuhnert and Linde[7].

Our experiments were carried out at room temperature in Petri dishes using a solution volume of 11 ml to give a layer ~1 mm deep. As the waves were hardly visible in daylight, they were observed and photographed under filtered blue light from a high-pressure mercury discharge lamp. The light intensity selected was low enough to prevent photochemical excitation of the reaction mixtures (formation of bubbles).

The reaction solutions were prepared in the following way. Mixtures of 7 ml of distilled water, 3 ml of sulphuric acid (4 M), and 1 ml of a solution of sodium bromate (1.5 M), were stirred and saturated with gallic acid; excess gallic acid was filtered off rapidly, and the filtrates were poured into Petri dishes. Reaction times were measured from when components were mixed. The brown solutions rapidly turned pale but darkened again slowly. After periods of 3 to 10 min colourless waves started propagating with a speed of 2 to 4 mm per minute from leading centres commonly situated at the borders of the dishes. In most experiments several leading centres appeared, and when the waves propagating from them came into contact, destructive interference was observed. Artificial leading centres could be created by adding droplets of concentrated sodium bromate solution to the reaction mixtures, these leading centres exhibited the same behaviour as the original ones, but often generating waves of shorter wavelength. Wave propagation was generally

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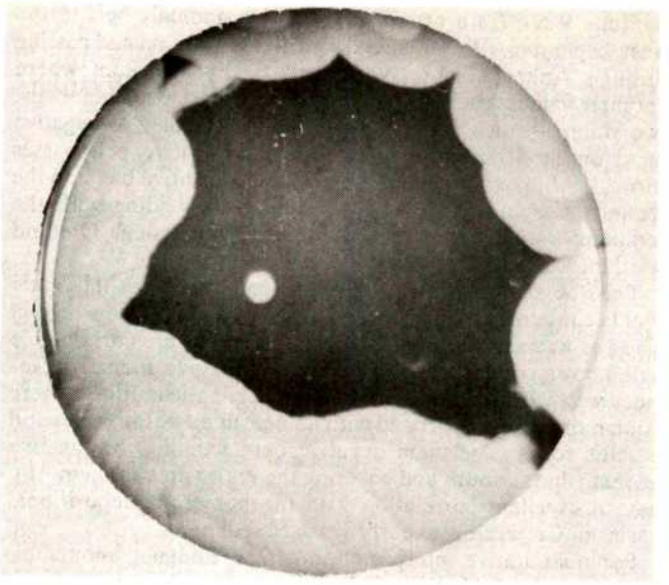


Fig. 1 Wave propagation in the uncatalysed gallic acid–bromate system. The diameter of the Petri dish is 100 mm. Concentrations (molar): H_2SO_4, 1.09; $NaBrO_3$, 0.136; gallic acid, ~0.04 (saturated solution). The reaction time of the mixture was 12 min 20 s. To avoid reflections, the dish was uncovered while the photograph was taken.

observed for periods of about 30 min, the waves fading slowly.

In several cases spatial irregularities were observed at the beginning of the reaction: areas not yet reached by a wave front suddenly showed an irregular colour change propagating about 10 times faster than wavefronts. This effect disappeared as soon as a wavefront reached these areas. The same effect was reported by Zaikin and Zhabotinskii[3]. We assume this effect to be a pseudo-wave which propagates much faster than ordinary trigger waves, and which appears only where trigger waves have not yet started propagating[9].

There has been some controversy as to whether those leading centres (also called pacemakers) from which the waves propagate may appear spontaneously. It arose because Winfree[10] assumed that in the BZR leading centres were caused by dust particles or similar heterogeneities of the system. Note that the general theory of irreversible thermodynamics forces an amplification of fluctuations in the system. An oscillatory reaction involves autocatalytic steps which are able to amplify such fluctuations.

Zaikin and Kawczynski[11] developed a theory to explain this effect. They distinguished between the pacemaker and the leading centre. The pacemaker is caused by a permanent local heterogeneity (such as a dust particle), whereas the leading centre can only be produced by an initial pertubation of concentration or phase. It would be very difficult to exclude completely all impurities, such as dust particles, and impurities may constitute a much greater potential disturbance of the homogeneity of the system than the fluctuations under study. Thus a macroscopic heterogeneity might be expected to trigger waves more effectively than can fluctuations. Similar earlier controversies concerned the oscillatory behaviour of the Bray–Liebhafsky reaction which was thought to be caused by gas bubbles.

We have given an additional example showing that a well defined macroscopic concentration gradient can also trigger chemical waves. It should be emphasised, however, that even when all impurities were excluded as far as possible wave propagation started spontaneously. Presumably the induction and synchronisation of the temporal and spatial phase difference in the propagating wave took a longer time in this case. The pseudo wave seems to be an early step in the development of this process.

The brown colour observed is probably caused by oxidative formation of a semiquinone compound from gallic acid, but the precise reactions that make up this oscillatory reaction system are not yet known.

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Gravity field of Benue Trough, Nigeria

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The Benue Trough, located at the Gulf of Guinea re-entrant on the West African coast (Fig. 1), is a unique Cretaceous rift whose origin is related to the opening of the South Atlantic and the continental separation of Africa from South America. The trough extends northeastwards for ~700 km from the Niger Delta to the Chad Basin and is filled with an estimated 5,000 m of Cretaceous sediments and volcanic rocks. Studies on the origin and tectonic evolution of the Benue Trough have been based so far principally on surface geological and geochemical evidence[1–5]. Gravity measurements by Cratchley and Jones[6] in the middle Benue area are of limited significance because they were aimed mainly at resolving the then controversy between the tensional and compressional[8] hypotheses. New and more extensive gravity data presented here indicate rifting in the upper and middle sections of the trough whereas the complex field over the lower section could be due to an 'unsuccessful' attempt to open into an ocean during the late Cretaceous.

Six hundred additional gravity measurements were made at 2–10 km spacings along passable roads (in the lower and upper areas of the trough) to fill up earlier gaps in information[9]. A Canadian Scintrex CG-2 gravimeter No. 305G, with a repeat accuracy of 0.01 mGal and average drift of 0.06 mGal h^{-1}, was used in measuring the gravity values. About 20% of the gravity stations were located on known elevation bench marks; elevations of other stations were determined by using a single Paulin system altimeter in loops of up to 2-h duration. These elevations have an estimated error of ±15 m. Station positions which have been established on a 1:1,000,000 scale map are correct to ±500 m. Free-air, Bouguer, terrain and latitude corrections were applied to the observed gravity values. The maximum possible error of the final Bouguer anomaly values is estimated to be about ±3 mGal.

Figure 2 is the resulting Bouguer contour map with relative gravity values ranging from −60 mGal near Kaltungo to +20 mGal near Abakaliki. Generally the Bouguer values decrease towards the flanking basement complex areas; for example, the value near Jos (Younger Granite province) is −90 mGal (ref. 9). Two distinct regions can be easily identified: (1) a positive gravity anomaly belt whose axis begins from south of Ishiagu, reaches +20 mGal near Abakaliki and terminates near Gboko. Beyond Gboko, the positive anomaly becomes much smaller in magnitude and disappears near south of Zurak

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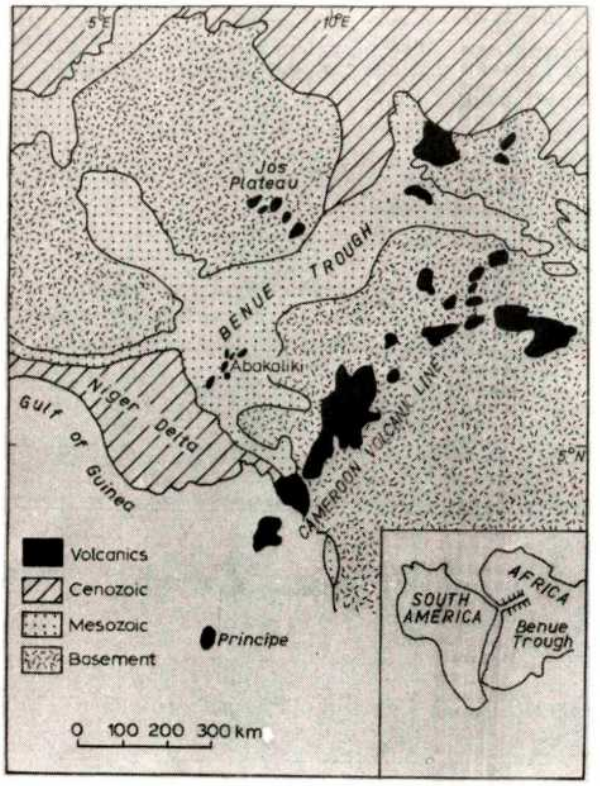


Fig. 1 Location map of Benue Trough.

(latitude 9°N); (2) a prominent negative anomaly belt to the west, beginning with −40 mGal south-east of Benin and passing through Ankpa, Wase, Wikki and finally Potiskum where anomaly values reach −60 mGal. Besides these two main belts, two others—much less defined—are discernible. A negative band on the eastern flank of the main positive belt passes through Afikpo, Ogoja and Wukari, and a positive band on the western margin of the map and almost coinciding with the sediment–basement boundary can be traced through Ore and south of Lokoja to Shendam.

The Bouguer map is relatively more complex south of latitude 9°N because of the complex geological structure of this region. The positive and negative Bouguer anomalies are correlatable with known surface geology; the belt of positive anomaly coincides well with the core of the Abakaliki anticlinorium where Albian shales are associated with numerous mafic intrusives and basaltic rocks. The main negative belt, flanking the positive anomaly in the south and covering the entire area in the north, has an excellent correlation with the numerous depositional basins of Cretaceous to early Tertiary sediments.

For quantitative interpretation of the Bouguer anomalies, four profiles (AA′, BB′, CC′, DD′) across the trough were selected (Fig. 2). The most outstanding feature of the profiles is a regional positive anomaly over the whole trough floor (Fig. 3). The profiles of the regional anomaly display striking similarity with one another, suggesting a deep-seated structure of considerable extent along the trough. Each profile in Fig. 3 also shows the uplift in the Moho boundary that would cause the gravity regional (with a mantle–crust density contrast of 0.57 g cm^{-3}). The mantle–crust interface (Moho) thus seems to have been elevated by ~1.5 km in the Lower Benue

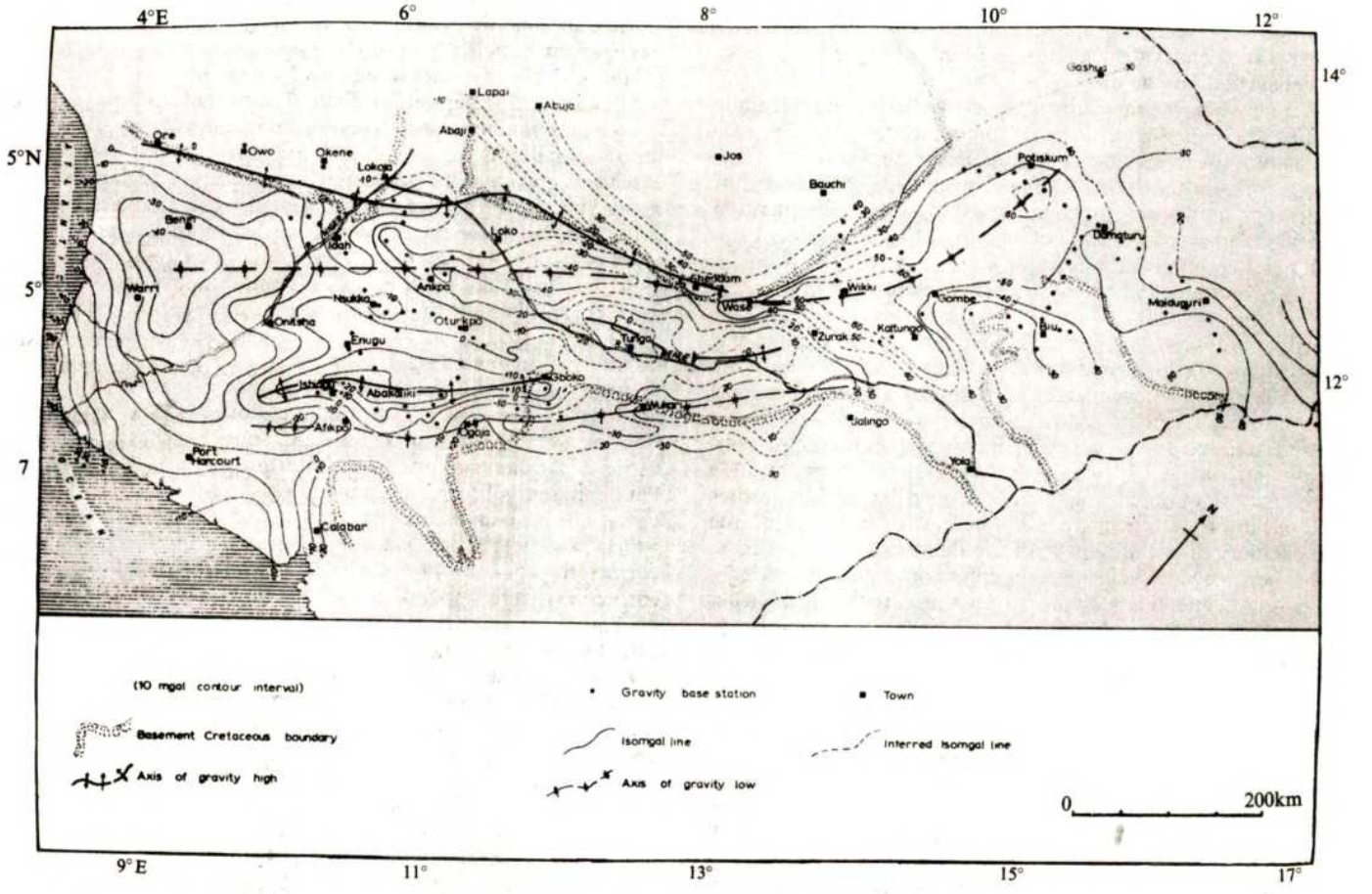


Fig. 2 Bouguer anomaly map of Benue Trough.

and ~3.0 km in the Upper. On the other hand, the width of the Moho uplift decreases from 170 km in the Lower to 70 km in the Upper Benue.

The residual positive anomaly belt from Abakaliki to Gboko has been interpreted as a two dimensional body of density 2.90 g cm^{-3}, average width of 30 km, and extending from a depth of 10 km down to the Moho (Fig. 4).

Uplifting of the Moho beneath rift structures has also been observed in other regions—Kenya Rift Valley[10], Gulf of Suez[11], Red Sea[12], Baikal Rift[13] and Oslo Graben[14]. Artsybash and Kogbe[15] had previously obtained a rather high value, 10–12 km, for the mantle uplift in Middle Benue. This is primarily due to their use of too small a value for the mantle–crust density contrast, namely, 0.17 g cm^{-3}, as against the 0.57 g cm^{-3} used here. The relatively small mantle uplift, as obtained in this study, could relate to the fact that the Benue Rift became inactive during the late Cretaceous following the rotation of the African plate and the consequent shift of tectonic activity to the Cameroun volcanic line[5].

The shape and size of the high density material that generates the main positive anomaly belt (Abakaliki–Gboko) is shown in Fig. 4. This anomalous body can hardly be considered as an intrusive because of its very large dimensions. It rather resembles a 'mantle diapir' which, in turn, might have been the source of the volcanic rocks in the area.

The negative anomalies which flank the positive belt in the south, and form the axial plane in the north, are considered to be due entirely to the sediments that fill the Benue Trough. The

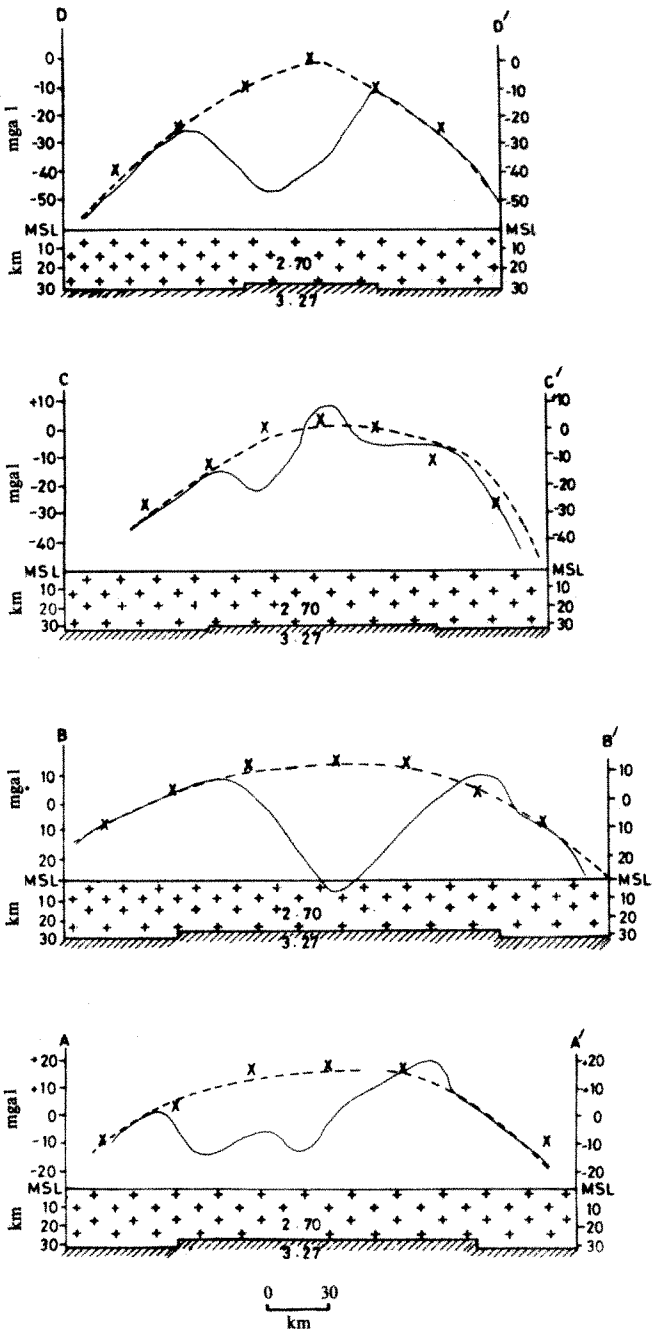


Fig. 3 Calculated regional anomaly for crustal models along profiles AA′–DD′. Solid line, observed gravity; dashed line, regional anomaly; ×, calculated anomaly due to the regional model.

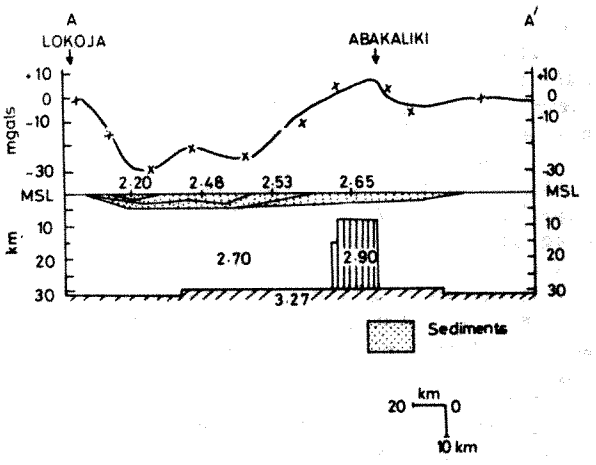


Fig. 4 Calculated residual gravity anomaly for model along profile AA′. Solid line, residual anomaly; ×, calculated anomaly.

maximum thickness of sediments in the Lower Benue area was calculated as 4,250 m (Fig. 4). In the Upper sections a maximum thickness of 6,000 m has been obtained[6].

The results of this study are consistent with a tensional origin related to the opening of the South Atlantic. The gravity anomaly patterns in the upper and middle sections of the trough are similar to those of other sediment-filled rifts. The complexity of the gravity field in the Lower Benue can be attributed to its proximity to the hotspot and triple junction which was beneath the present Niger delta. This was associated with an unsuccessful attempt to open the Benue Trough into an ocean during the late Cretaceous.

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Evidence for organically associated iron in nearshore pore fluids

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The role of dissolved organic matter (DOM) in the speciation of trace metals in natural waters is poorly understood[1-3]. Although dissolved organic matter–iron associations have been observed in freshwater systems[4-6], it has been thought that DOM–iron species are not significant in saline waters[7]. Yet recent studies have suggested that iron–DOM interactions in the pore waters of anoxic marine sediments may be important[8,9]. The possibility of organically associated trace metals in anoxic water has been used to explain why metal concentrations are higher than those predicted by sulphide-mineral equilibria[10,11]. We present here data that indicate that strong iron–DOM associations do occur in the anoxic pore waters of nearshore marine sediments and that both the total dissolved iron and organically associated iron change seasonally.

Five box cores were taken within 1 m^2 of each other between February and September 1978, in a subtidal area in the Great Bay estuary, New Hampshire. The cores were treated very carefully so that the sediment and its pore fluid would not be exposed to the atmosphere at any time. In the laboratory the corer was placed in a nitrogen-filled glove bag, extruded, sectioned into 2-cm sections, centrifuged and filtered through precleaned 0.4-μm membrane filters. Details on handling and cleaning procedures are published elsewhere[12,13]. The inert atmosphere was maintained to minimise oxidation artefacts[14-16]. While in the glove bag, the filtered pore water samples were separated into two aliquots and one acidified to pH ~1 with ultrapure HNO_3 and the other acidified to a pH of ~2.8 with ultrapure HCl. The HNO_3 acidified samples were then placed into a water bath at 90 °C, heated for 1 h and analysed for total dissolved iron using the technique of Murray and Gill[17]. The HCl acidified samples were passed through a column of the macroeticular resin, Amberlite XAD-2, utilising the flow parameters outlined by Mantoura and Riley[18]. This resin has been shown to be very efficient in removing humic substances from natural water samples of pHs between 2 and 3 (refs 19–21). Due to the harshness of this procedure (acidification), the Amberlite extractable iron fraction should be considered as iron that is very strongly associated with the humic material present in the pore fluids. Sugimura *et al.*[22] have shown, however, that 80–90% of the total iron present in seawater is removed by procedures similar to those outlined above. Only 10% of the total iron is removed by the resin if the sample is not previously acidified. This technique probably underestimates the iron that is actually associated by pore water DOM. The resin was eluted with ultrapure 1M NaOH, acidified to pH ~1 with ultrapure HNO_3, heated in the water bath for 1 h and analysed for 'humic-substance associated iron' or organic iron. The standard deviation of the iron measurements reported as a coefficient of variation was 2% at 10 μmol^{-1} and 10% at 2 μmol^{-1}. The data are shown in Table 1.

The total dissolved iron values proceed through a seasonal cycle with the highest concentrations observed in the late spring and the lowest in mid-summer. This seasonal cycle of dissolved Fe can be correlated with bacterial cycling of sulphur and DOM in these sediments[23]. In the spring, as the temperature of the water and the sediment increases, anaerobic fermenting bacteria begin to metabolise at a faster rate producing reduced end-products which lower the sediment Eh. As the Eh is lowered more Fe^{3+} is solubilised to Fe^{2+} in the pore waters. Bacterially mediated sulphate reduction occurs as the temperature increases and the Fe present in the pore fluids in the late spring is removed by mid-summer by the *in situ* formation of FeS. Similar seasonal pore cycling of Fe has recently been observed in temperate salt marsh sediments[24].

The May samples also have the highest percentages of organic iron in the pore waters, with an average of 31% of the total iron being organically associated. This compares with 10% or less for the other sampling dates. It thus seems that the production and removal of this organic-iron fraction is strongly related to sedimentary microbial dynamics. Laboratory experiments have shown that the pigment and lipoidal fractions (1,500–50,000 molecular weight fraction) of DOM from the bacterial degradation of algae can form water soluble iron-organic associations[25]. It has been suggested that a syntrophic relationship exists between fermenting and sulphate reducing bacteria and that fermenting bacteria produce organic substrate for subsequent sulphate reducing bacterial growth[26]. Although our conclusions are based on a single set of samples from one site, it seems that this interaction between bacterial groups may also have a profound effect on trace metal speciation in anoxic waters.

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Table 1 Pore water concentration of total dissolved iron and organically associated iron from Great Bay, New Hampshire

Depth (cm)	10 February		27 March		Fe (μmol^{-1}) 9 May		20 July		22 Sept 78	
	Total	Organic	Total	Organic	Total	Organic	Total	Organic	Total	Organic
1	50	<1	39	1	220	50	<1	<1	54	2
3	—	—	50	2	119	15	<1	<1	21	2
5	100	1	19	3	101	20	<1	<1	10	1
7	69	8	18	1	40	10	<1	<1	1	<1
9	34	3	8	1	37	10	<1	<1	3	<1
11	12	2	8	1	9	7	<1	<1	2	<1

All measurements were taken in 1978.

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Cell-surface labelling reveals no evidence for membrane assembly and disassembly during fibroblast locomotion

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As a cultured fibroblast moves, particles or pieces of debris attached to its surface move backwards with respect to both the cell and the substrate[1,2], as also do concanavalin A (Con A) receptors in the membrane of the leading lamella[3]. One suggested explanation is that membrane components from the cytoplasm are assembled and introduced into the surface membrane of the moving cell close to its leading edge and flow backwards to a 'sink'; there the membrane is disassembled, and its components enter the cytoplasm and flow forward within the cell, to be re-introduced later into the surface membrane[1,4]. However, because cell-surface receptors can be redistributed as a result of cross-linking by external ligands[5,6] it has been proposed that the backward flow of Con A receptors and particles may result from such a cross-linking rather than from a flow of intact cell membrane[7,8]. To investigate these alternatives, I have studied moving fibroblasts by means of a cell membrane label that does not induce the redistribution of its receptors. My results do not seem compatible with the membrane flow model.

The Thy-1.1 alloantigen (formerly ΘAKR) is an integral membrane glycoprotein originally identified on mouse T lymphocytes[9] and also found on fibroblasts from Wistar–Furth (W/Fu) rats[10,11]. Treatment of lymphocytes[12] and W/Fu fibroblasts (C.A.M., unpublished observations) with alloantiserum directed against this alloantigen (anti-Thy-1.1) does not result in the redistribution of the antigen. This does not imply, however, that the antigen is anchored in the membrane, for in lymphocytes[12], and both suspended and spread W/Fu fibroblasts (C.A.M., unpublished observations), incubation with an additional layer of antibody directed against anti-Thy-1.1 will induce the redistribution of the antigen into patches and caps.

Cultured rat W/Fu fibroblasts were cooled rapidly to inhibit locomotion and incubated with mouse anti-Thy-1.1 in the cold. The cells were then returned to 37 °C and the locomotion of a selected cell was filmed: ruffling and locomotory activity of the cells was unaffected by incubation with anti-Thy-1.1. The cells were again cooled to prevent further locomotion, and before fixation were incubated with fluorescein-conjugated rabbit anti-mouse IgG (anti-MIg-Fl) to make possible visualisation of the anti-Thy-1.1 (details in Fig. 1). The selected cell was then observed by incident light fluorescence microscopy.

When there was no incubation at 37 °C, so that cells were unable to move between treatments with the two antisera, there was an even distribution of fluorescent label (Fig. 1), indicating

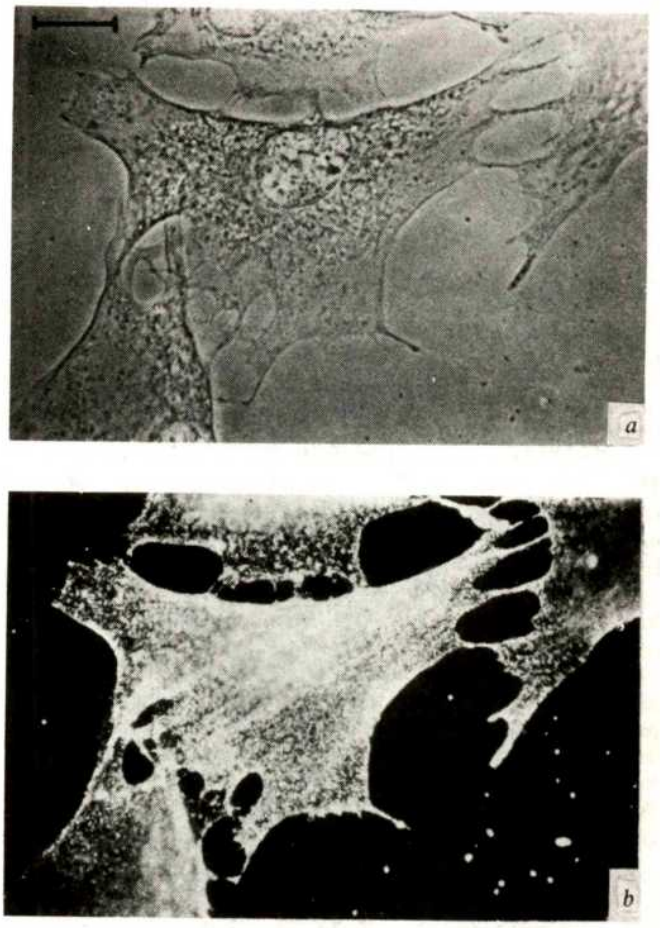


Fig. 1 Fluorescent labelling of Thy-1.1. Fibroblasts were initially obtained as contaminants of myoblast cultures from neonatal W/Fu rats. They were subsequently maintained by twice weekly subculture in Dulbecco's modified Eagle's medium (MEM) containing 10% fetal calf serum (FCS, Flow Labs), penicillin (100 IU ml^{-1}) and streptomycin (100 $\mu g\ ml^{-1}$). When required, cells were collected and adjusted to a concentration of $5\times10^3\ ml^{-1}$ in medium as above, and 1-ml aliquots of this suspension were plated out on to 13-mm diameter glass coverslips contained within Linbro multidish wells (16 mm diameter, Flow Labs). The coverslips had previously been marked, using Indian ink, with a reference grid of side approximately 2 mm to allow the subsequent localisation and identification of selected cells. After 24 or 48 h culture, a selected coverslip was cooled rapidly by washing in HEPES-buffered MEM (MEM-H) containing 20% FCS at 4 °C and then incubated in anti-Thy-1.1 (1:4) prepared as described by Raff[18] for 30 min at 4 °C. The coverslip was then thoroughly washed in MEM-H at 4 °C and maintained in MEM-H plus 10% FCS at 4 °C, and a suitable cell selected. The cold medium was then replaced with MEM-H plus 10% FCS prewarmed to 37 °C and the selected cell was filmed under phase contrast optics by time-lapse cinematography (1 frame per 3 s) for 15–180 min. At the end of the period of filming, the culture was rapidly cooled by washing in MEM-H plus 20% FCS at 4 °C and then incubated in anti-MIg-Fl (1:10, Miles Labs) for 30 min at 4 °C. After thorough washing in MEM-H at 4 °C, the culture was fixed in absolute methanol for 2 min and mounted in phosphate-buffered saline. The culture was examined using a Leitz Orthoplan microscope equipped with phase contrast and incident light fluorescence optics suitable for fluorescein fluorescence. The filmed cell was re-identified using the reference grid and paired phase contrast and fluorescence micrographs obtained. *a*, *b*, Distribution of fluorescence on control cells. The period of locomotion at 37 °C was eliminated from the above protocol by maintaining the cells at 4 °C throughout; incubation with anti-Thy-1.1 was followed immediately by washing and incubation with anti-MIg-Fl. A group of cells was photographed with phase contrast optics (*a*) and with incident light fluorescence optics (*b*). Scale bar, 20 μm.

an even distribution of Thy-1.1 over the surface of the cell. Fluorescent labelling was prevented if there was no incubation with anti-Thy-1.1.

Figure 2 shows an experiment in which a selected cell was filmed for 30 min at 37 °C after incubation with anti-Thy-1.1. The cell ruffled during filming and stills taken from the first (Fig. 2*a*) and last (Fig. 2*b*) frames of the film show that regions of the leading lamella advanced up to 30 μm (Fig. 2*c*). Treatment with anti-MIg-Fl showed that the distribution and intensity of the

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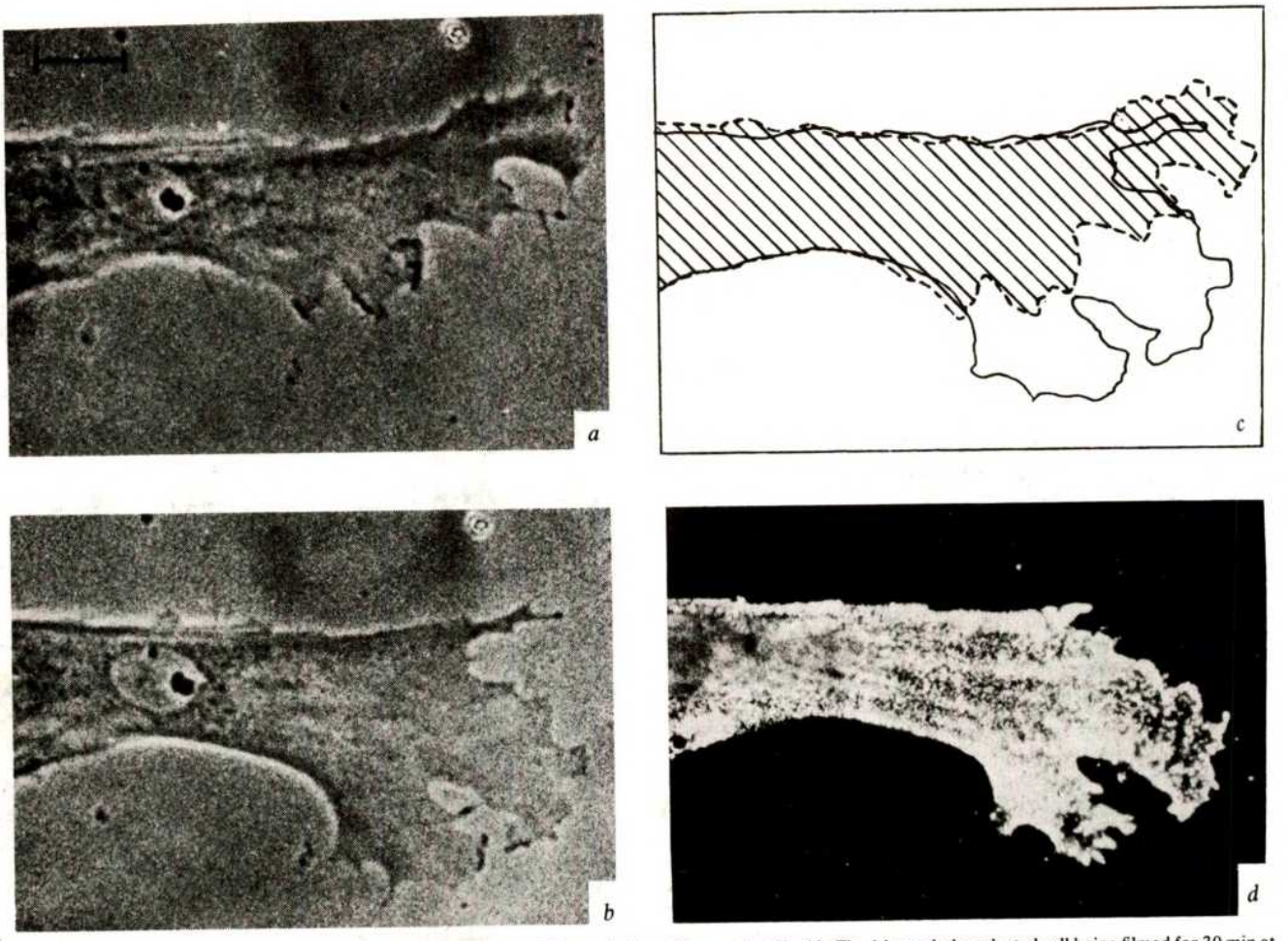


Fig. 2 Distribution of fluorescence on a fibroblast after locomotion. The method used was as described in Fig. 1 legend, the selected cell being filmed for 30 min at 37 °C after incubation with anti-Thy-1.1 and before incubation with anti-MIg-Fl. *a*, *b*, Stills taken from the time-lapse film at the start of filming (*a*) and at the end of filming (*b*); *c*, superimposition of tracings from *a* and *b* to show the movement of the cell; *d*, fluorescence micrograph of the same cell. Scale bar, 20 μm.

fluorescent labelling (Fig. 2*d*) were indistinguishable from that on control cells which had not moved (compare Figs 1*b* and 2*d*). Similar experiments in which the cells moved for 15–180 min after incubation with anti-Thy-1.1 antiserum yielded identical results.

Con A labelling experiments had suggested a coordinated backward flow of newly assembled membrane from the leading edge of the cell[3], but my results are incompatible with that. If membrane were assembled during the period of locomotion, the newly assembled membrane would contain Thy-1.1 previously within the cytoplasm, unavailable to the anti-Thy-1.1 and therefore not detectable by anti-MIg-Fl. A coordinated backward flow of newly assembled membrane would therefore appear as a gradual retreat of fluorescent label from the leading edge, because, with increasing time, more and more membrane containing Thy-1.1 unavailable for subsequent labelling would be introduced at the leading edge of the cell. There was no sign of such a retreat.

Although my results argue against coordinated backward flow of the membrane, some less organised flow may be possible because membrane components, both protein and lipid, are free to diffuse in the plane of the membrane (see ref. 6 for review), and so components inserted during locomotion might be expected to mix with those already present on the cell surface. In my experiments any Thy-1.1 inserted during locomotion is unavailable for subsequent labelling (see above). This will mix with and dilute the Thy-1.1 already present on the surface and available for labelling at a rate largely determined by the speed of membrane assembly and disassembly. This dilution would be detected as a gradual decrease, with time, in the intensity of subsequent labelling. Studies of particle movements during fibroblast locomotion suggested that the membrane was being assembled and disassembled at a rate equivalent to replacing the entire surface of the cell approximately once every 10 min (ref. 2). In my experiments, such a rate should have led to a rapid decrease in the intensity of labelling with increasing periods of locomotion. I have not estimated fluorescence quantitatively, but qualitatively no such decrease was observed. Cells that had been allowed to move for periods of up to 180 min were apparently as fluorescent as control cells that had not moved and it was still possible to label fluorescently cells that had been maintained at 37 °C for 24 h after incubation with anti-Thy-1.1.

In considering the dilution of the cell-surface label in this way I make two assumptions. First, if the membrane is disassembled, Thy-1.1/anti-Thy-1.1 complexes will be degraded and cannot be reintroduced into the membrane for subsequent re-labelling. Second, at the proposed membrane sink, anti-Thy-1.1 is not released from the Thy-1.1 antigen and is therefore not available to diffuse through the medium to bind to uncomplexed Thy-1.1 antigen.

Both assumptions seem justified, as membrane-bound antibodies are usually endocytosed, enter the lysosomal system and are degraded[13,14]. Membrane-bound cationised ferritin has been reported to enter secretion granules and Golgi cisternae[15], but even there it had often lost its association with the membrane[15]. Even if the second assumption was incorrect and anti-Thy-1.1 was released at the sink, its recomplexing with unoccupied Thy-1.1 would have to be unexpectedly efficient to have maintained the observed levels of fluorescence, in view of the predicted rates of membrane assembly and disassembly and its likely rate of diffusion in the culture medium.

It seems unlikely from the results presented here that cell locomotion involves the assembly and disassembly of membrane components at the rate predicted by the membrane flow model.

Probably the backward movement of Con A receptors, and possibly also that of particles, during locomotion, results from a ligand-induced redistribution of membrane components. However, it is difficult to account for the commonly observed [16] backward movement of blebs and ruffles in this way. The movement of these membranous organelles suggests that some backwardly directed force is generated on the surface of moving cells, but it seems unlikely to be due to the flow of intact membrane. Conceivably, the proposed flow of the membrane lipids alone[17] could generate such a force, but this flow remains to be demonstrated.

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Genetic polymorphism of human complement C4 and detection of heterozygotes

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Many components of complement exhibit inherited structural polymorphism, with at least two alleles being common in one or more of the major races of man[1–9]. However, definition of genetic polymorphism in human C4 has proved a difficult task. Differences between the electrophoretic patterns of C4 in EDTA plasma from different individuals were detected by Rosenfeld and coworkers[10,11], but these differences could not be explained by any simple genetic model. O'Neill and coworkers[12], using a system similar to that of a previous study[13], interpreted the electrophoretic patterns in a completely different way from previous workers[13,14], stating that there were three: F, FS and S. They also found that C4 F carried Rodgers or Rg(a+) serologic reactivity and C4 S carried Chido or Ch(a+) reactivity[15]. These 'blood group' antigens had previously been shown to be closely linked to HLA[16,17]. O'Neill proposed[15] that C4 is genetically controlled by two closely linked loci, one for C4 F and one for C4 S, and postulated that deficiency states for one or the other locus are common but rarely occur together. The sets of haplotypes were postulated to be *C4 Fs*^{0} (or *C4 F*), *C4 f*^{0}*S* (or *C4 S*) and *C4 FS*. In this model, the C4 F individuals are Rg(a+)Ch(a−), C4 S individuals are Rg(a−)Ch(a+) and C4 FS persons are Rg(a+)Ch(a+). The chief problem with the methods of typing, both electrophoretic and serologic, is that *C4 Fs*^{0} and *C4 f*^{0}*S* can usually only be detected in the homozygous state, thus severely limiting their usefulness. We have now developed a method of C4 typing that allows detection of *C4 Fs*^{0} and *C4 f*^{0}*S* in heterozygotes, and permits the study of genetic polymorphism of *C4* in the general population. Our observations confirm and extend the two-locus genetic model of O'Neill and coworkers.

Five kinds of patterns were found using samples from randomly selected caucasians, as shown in Fig. 1: (1) F = S, (2) S only, (3) F only, (4) F > S, (5) F < S. When the stained crossed immunoelectrophoresis patterns were subjected to quantification of the ratio between the F and S peaks by placing the patterns over 1-mm graph paper and counting squares, for F > S, F/S was 219 ± 25% (±1 s.d.) for $n = 10$ and for F < S, F/S was 52 ± 7% for $n = 10$. For F = S, the ratio was 96 ± 8%, with $n = 13$. Thus, patterns (2) and (3) are, as described by O'Neill and coworkers[15], from individuals who are *C4 f*^{0}*S/f*^{0}*S* and *C4 Fs*^{0}*/Fs*^{0}, respectively, and patterns (4) and (5) are from individuals predicted by them to occur but not distinguishable by their typing system, *C4 Fs*^{0}*/FS* and *C4 f*^{0}*S/FS*. Pattern (1) is the only ambiguous phenotype, as it would be given by both *C4 FS/FS* and *C4 f*^{0}*S/Fs*^{0} individuals.

The concentrations[18,19] of C4 in the plasma (as per cent normal) of various C4 types are: for 32 *FS/FS* individuals, 109 ± 44% (±1 s.d.); for 24 *Fs*^{0}*/FS* individuals, 101 ± 48%; for 9 *Fs*^{0}*/Fs*^{0} individuals, 75 ± 24%; for 18 *FS/f*^{0}*S* individuals, 100 ± 56%; for 4 *f*^{0}*S/f*^{0}*S* individuals, 70 ± 24%; and for 7 *Fs*^{0}*/f*^{0}*S* individuals, 67 ± 24%. It is of interest that the means of C4 serum concentration in individuals of different C4 types differed, as expected. However, there was considerable overlap,

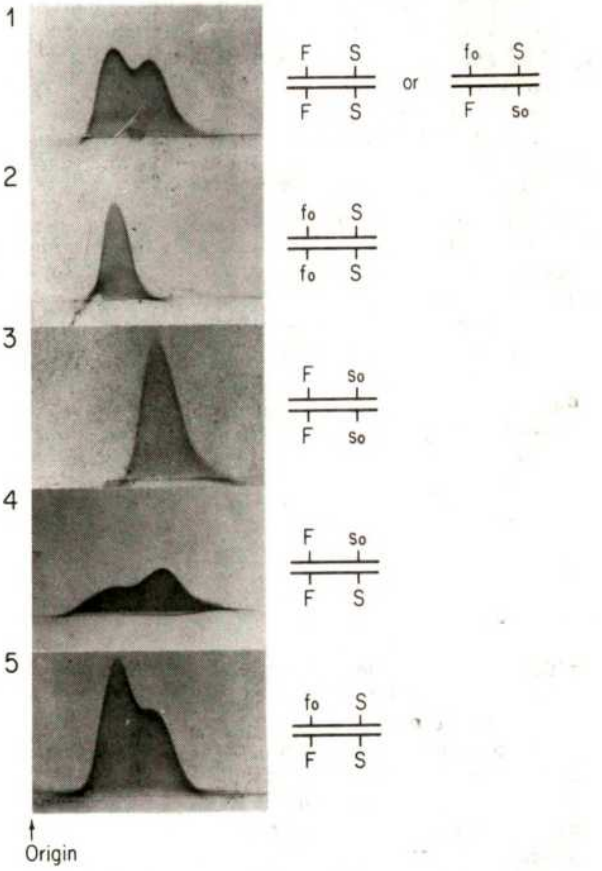


Fig. 1 Crossed immunoelectrophoresis patterns for the five detected phenotypes of C4. Fresh plasma from whole blood collected into Na_2 EDTA at 1.1 mg ml^{-1} was used immediately or stored at −80 °C and thawed just before analysis. The plasma samples were incubated with neuraminidase (Sigma) from *Clostridium perfringens* at 5 U ml^{-1} for 15 h at room temperature while dialysed against 0.1 M phosphate buffer, *p*H 7.0, containing 0.005 M Na_2EDTA. Desialated plasma samples were then subjected to electrophoresis in 0.8% agarose (Marine Colloids) using 0.075 M Veronal buffer containing 0.005 M EDTA at *p*H 8.6 for both gel and vessels. Electrophoresis was carried out at 300 V and 50 mA for 3 h by which time a HbA marker had migrated 6.0 cm. Gel strips (3 mm wide) containing separated proteins and extending from the origin to the HbA area were transferred to a second agarose gel containing goat anti-human C4 (Atlantic Antibodies) at 1% and then subjected to crossed immunoelectrophoresis[25]. In a few instances, immunofixation[26] with anti-C4 was used after the first separation.

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so that serum C4 concentrations were of little use in distinguishing C4 types, including *C4 FS/FS* from *C4 f⁰S/Fs⁰*, or even *C4 Fs⁰/Fs⁰* and *C4 f⁰S/f⁰S* from *C4 FS/FS*.

When the frequencies of haplotypes *FS*, *f⁰S* and *Fs⁰* were calculated in a population of 203 randomly selected caucasians assuming all F = S patterns to be of genotype *FS/FS*, the frequencies of *FS*, *Fs⁰* and *f⁰S* were, respectively, 0.74, 0.14 and 0.12. These frequencies were not significantly different from those predicted by the Hardy–Weinberg equilibrium ($P = 0.54$). The frequency of *FS* is overestimated (by about 3%) and the frequencies of *f⁰S* and *Fs⁰* are underestimated. Evidence for the autosomal codominant inheritance of these *C4* genes was obtained in family studies, as shown in Table 1 in which only expected types were found in offspring of various mating pairs, and the offspring types and ratios were close to those predicted. Inheritance of *C4* haplotypes for three families is illustrated in Fig. 2.

Although the genetic model for *C4* polymorphism in man as suggested by O'Neill and coworkers[15] explains some of the previously confusing and conflicting observations, the problem of detecting the various proposed *C4* 'haplotypes' in their heterozygous combinations remained. In our experiments, overlap between C4 F and C4 S occurred in all existing separation systems, including isoelectric focusing in polyacrylamide gel. O'Neill and Dupont[20] attempted to identify *C4 FS/f⁰ S* and *C4 FS/Fs⁰* patterns based on the intensity of immunofixation bands in their system. By comparison with frequencies predicted by the Hardy–Weinberg equilibrium and their observed homozygote frequencies, they were able to detect less than 20% of predicted heterozygotes. For these reasons we used neuraminidase treatment to reduce heterogeneity of each of the two *C4* locus products. This resulted in the ability to separate and quantify C4 F and C4 S by ordinary agarose gel electrophoresis, thereby permitting *C4* genotypes to be determined in random individuals and families. Thus, it has become feasible to demonstrate the inheritance of *C4* polymorphism by classic mendelian genetics.

The only remaining ambiguity in determining *C4* haplotypes is the distinction between *C4 FS/FS* and *C4 f⁰S/Fs⁰* ($F = S$), much like AA and AO blood group genotypes manifested by the single phenotype, group A. This ambiguity could usually be resolved by family studies (see family I, Fig. 2). Homozygous C4 deficiency[21,22] has been found in only six individuals and is thus extremely rare. There may also be ambiguity in the instance where the rare, presumed haplotype for this deficiency, *C4 f⁰s⁰*, occurs in an individual with a single expressed structural haplotype. This problem, of course, is found in any genetically polymorphic system where a silent gene or haplotype is present. In this case, it is possible that 'anomalous' inheritance of structural types occurs.

In the mouse, a duplication of the *C4* locus, *Ss* and *Slp*, within the *H-2* region, also seems to occur, although one of these proteins, Slp, is not functional[23]. In the guinea pig, only one genetic locus has been detected for C4 (ref. 24). In man, C4 patterns are due to the products of two loci in which the two genes are probably so closely linked that crossing over between them is an unusual event. This and the ability to detect heterozygotes of all possible haplotypes will eventually allow proper segregation and linkage analysis for *C4* especially with regard to the genetic loci for the complement proteins, C2 and Bf, and the HLA complex.

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Table 1 Segregation of *C4* types in families*

Mating	No. of matings	No. of offspring	Phenotypes of children: FS/FS	FS/f⁰S	FS/Fs⁰	Fs⁰/Fs⁰	f⁰S/f⁰S	Fs⁰/f⁰S
FS/FS × FS/FS	3	12	12 (12)					
FS/FS × FS/Fs⁰	5	15	8 (7.5)		7 (7.5)			
FS/FS × FS/f⁰S	3	9	3 (4.5)	6 (4.5)				
FS/f⁰S × FS/f⁰S	1	8	2 (2)	5 (4)			1 (2)	
FS/Fs⁰ × FS/Fs⁰	1	5	4 (1.25)		0 (2.5)	1 (1.25)		
FS/Fs⁰ × FS/f⁰S	1	4	0 (1)	1 (1)	2 (1)			1 (1)
FS/Fs⁰ × Fs⁰/Fs⁰	2	10			5 (5)	5 (5)		
FS/f⁰S × Fs⁰/f⁰S	1	4					2 (2)	2 (2)
FS/f⁰S × Fs⁰/Fs⁰	1	5			2 (2.5)			3 (2.5)

* By χ^2 analysis, expected (given in parentheses) and observed types in children did not differ significantly.

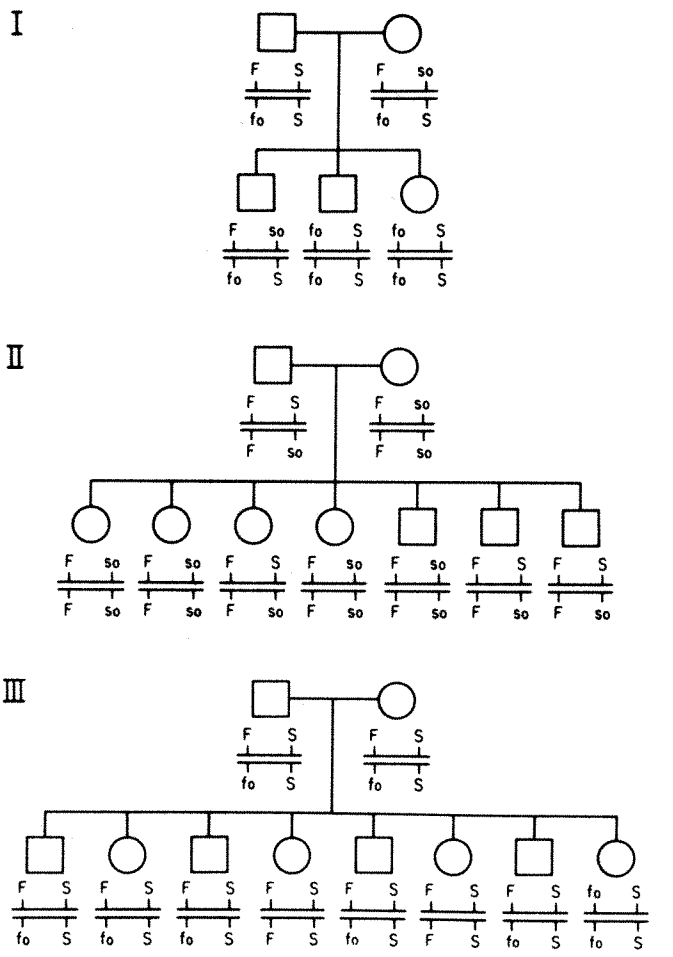


Fig. 2 Inheritance of *C4* haplotypes in three families.

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Genetic defect in secretion of complement C5 in mice

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A genetic deficiency of the fifth (C5) component of complement[1–3], a serum glycoprotein of molecular weight (MW) 220,000 (ref. 4), has been found in 39% of inbred strains of mice[3]. Sera of deficient mice lack detectable C5 activity and protein[2,3]. In addition deficient mice produce antibody to mouse C5 when injected with sera from C5 sufficient (normal) strains. Levy *et al.*[5] showed that somatic cell hybrids between C5 deficient (B10.D2/old line) macrophages and either C5 sufficient (B10.D2/new line) mouse kidney or chicken erythroblasts secreted haemolytically active mouse C5 *in vitro*. Several possible molecular mechanisms to account for the findings were considered, but insufficient direct data were available to choose among them. We recently reported that mouse (CD.1 strain) peritoneal cells in culture synthesise and secrete a single chain precursor, pro-C5 (MW ~ 210,000), of the two-chain (α chain, 125,000 and β chain 83,000 MW) C5 protein[6]. Radiolabelled precursor C5 was contained within the cells and was secreted into the tissue culture media. Using similar methods, we now find that C5 deficiency in each of five different mouse strains (AKR, SWR, DBA/2J, A/HeJ and B10.D2/old line) is due to a failure in secretion of C5 protein and not to a failure in biosynthesis of pro-C5.

Resident peritoneal macrophage cultures from five mouse strains genetically C5 deficient and from two normal (Swiss and B10.D2/new line) strains were established and pulsed with medium containing ^{35}S-labelled methionine. After 24 h, newly synthesised intracellular (cell lysate) and extracellular protein were quantitated by trichloroacetic acid (TCA) precipitation, specific C5 antigen by immunoprecipitation and C5 function in the extracellular medium by haemolytic assay. The size and subunit composition of the radiolabelled C5 protein were determined on SDS-polyacrylamide gel electrophoresis in reducing conditions.

Total protein (TCA-precipitable radioactivity) was similar in C5-deficient and normal cells in both the intracellular and extracellular compartments (Table 1). ^{35}S-methionine was incorporated into C5 antigen contained intracellularly and was secreted by normal peritoneal cell cultures. Although peritoneal cells from the deficient strains synthesised an amount of immunochemically detectable C5 protein similar to that produced by the normal strains, all of the C5 antigen was contained in the cell lysates (Table 1). In addition, approximately 2.5×10^8 haemolytically active C5 molecules ml^{-1} were present in the normal peritoneal cell culture media, but none was detected in any of the deficient cell culture media (limits of detection <2% of normal).

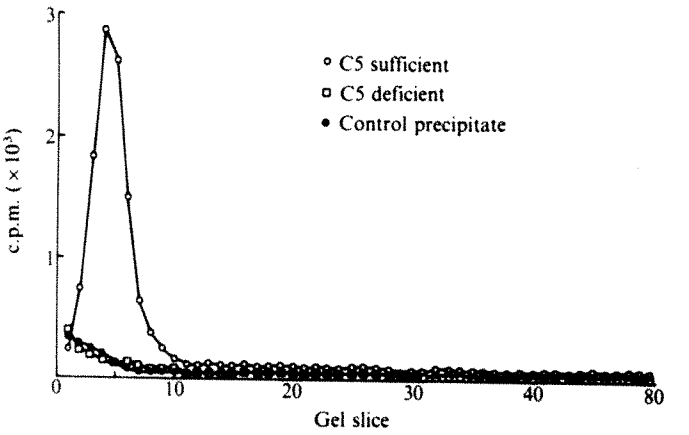


Fig. 1 SDS-polyacrylamide gel electrophoresis of reduced and alkylated C5 protein secreted by mouse peritoneal macrophages from Swiss (C5 sufficient) and A/HeJ (C5 deficient) strains. Confluent resident peritoneal cell cultures were established by placing 12×10^6 cells suspended in Dulbecco's modified Eagle's medium (DMEM) with 20% heated fetal calf serum in 60 mm Falcon plastic dishes. After incubating the cells at 37 °C for 1 h in humidified 5% CO_2 : 95% air to permit adherence, the dishes were washed with DMEM, then fed with DMEM (lacking unlabelled methionine), containing ^{35}S-methionine (80 μCi ml^{-1}), 50 mM ε-aminocaproic acid, 0.2% lactalbumin, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. The medium was removed 24 h later, fresh medium containing ^{35}S-methionine was added, and the cultures were incubated for a further 24 h. Medium was collected and centrifuged at 10,000*g* for 10 min to remove floating cells and debris. The cell monolayer was washed three times in phosphate-buffered saline, then lysed in Tris-KCl with 0.01 M Na EDTA, 0.1 M ε-aminocaproic acid, and 0.5% deoxycholate, and 0.5% Triton X-100 buffer[6], then centrifuged at 10,000*g* for 45 min. Total labelled protein was assessed by TCA precipitation of 5-μl aliquots (Table 1). Antiserum to mouse C5 was produced in C5 deficient (B10.D2/old line) mice by immunisation with normal (B10.D2/new line) mouse serum in complete Freund's adjuvant[2,6]. Specific C5 and control (ovalbumin) immunoprecipitates were formed as previously described[6,7], using unlabelled carrier proteins and antisera sufficient to yield 30 μg protein in the precipitates. They were washed, dried and then dissolved by boiling for 2 min in 40 μl buffer consisting of 10 mM Tris, 1 mM EDTA, 1% SDS, 4% 2-mercaptoethanol, 10% glycerol and Pyronine Y. Iodoacetamine (60 mM final concentration) was added and the mixtures incubated at 37 °C for 30 min. Aliquots of 5 μl were removed for estimates of specific incorporation of labelled methionine into C5 antigen (Table 1) and the remainder was applied to 5.6% polyacrylamide, 1% SDS disc gels. Electrophoresis, slicing and counting of samples and analysis of standard gels were performed as previously described[6,7]. Radioactivity in C5 antigen from C5-sufficient (Swiss) cell culture medium (○), C5-deficient (A/HeJ) cell culture medium (□) and control immunoprecipitates (●) are plotted. Peak of radioactivity (○) corresponds to estimated MW of 220,000.

Analysis of the C5 protein produced by normal cells on SDS-polyacrylamide gel electrophoresis showed a single peak of radioactivity (mobility corresponding to MW 220,000) in the extracellular medium (open circle, Fig. 1) and two peaks (MW 201,000 and 34,000) in the cell lysate (open circle, Fig. 2), in agreement with previous findings[6]. Radioactivity in control immunoprecipitates (ovalbumin/anti-ovalbumin) distributed uniformly throughout the gels without recognisable peaks (closed circles). No peak of immunoprecipitable C5 radioactivity was detected extracellularly (open squares, Fig. 1) in any of the C5-deficient cell cultures, but a pattern comparable to the normal was observed in the intracellular compartment (MW 200,000 and 38,000 peaks) (open squares, Fig. 2). Figures 1 and 2 show the result with A/HeJ cells; similar patterns were seen on analysis of the other deficient cell culture media and lysates. Following a 24-h pulse with labelled methionine, a 48-h

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Table 1 Synthesis and secretion of C5 and total protein by mouse peritoneal macrophages

Strain	Serum	C5 antigen: Extracellular (c.p.m.)	C5 antigen: Intracellular (c.p.m.) High MW	C5 antigen: Intracellular (c.p.m.) Low MW	Total protein c.p.m. ($\times 10^{-3}$): Extracellular	Total protein c.p.m. ($\times 10^{-3}$): Intracellular
A/HeJ	−	−115	970	2,700	3.5	74.9
AKR	−	50	1,600	2,960	5.1	100
B10.D2/old	−	−5	2,080	4,220	8.2	101
DBA/2J	−	−170	3,430	5,730	12.8	129
SWR	−	80	3,600	7,900	8.9	168
Swiss	+	1,820	2,030	3,380	8.2	100

Sera from mice used for peritoneal macrophage cultures were tested for the presence of C5 antigen by Ouchterlony analysis (results in agreement with findings in ref. 3). Peritoneal cell cultures were established as described in Fig. 1 legend and the extracellular C5 antigen estimated in 125-μl aliquots by subtracting the radioactivity in control (ovalbumin/anti-ovalbumin) immunoprecipitates (mean 427 c.p.m. per 125 μl; range 523 for B10.D2/old line to 308 for AKR) from radioactivity in C5-anti-C5 immunoprecipitates. Intracellular C5 antigen content was estimated from the radioactivity in the high MW pro-C5 peak and low MW peak on SDS-polyacrylamide gel electrophoresis of C5 immunoprecipitates (representative pattern is shown in Fig. 2). To estimate total protein synthesis, TCA precipitation was performed on 5-μl aliquots as described in Fig. 1 legend.

chase of C5-deficient (A/HeJ) cells with medium containing unlabelled methionine resulted in little change in the intracellular C5 antigen pattern on SDS-polyacrylamide gel electrophoresis and no appearance of labelled C5 protein in the extracellular medium. Estimation of pool size and intracellular turnover of C5 antigen cannot be determined, but within the time limits of the experiments, even delayed secretion of labelled C5 antigen was ruled out.

The possibility was considered that macrophages from the deficient strains secreted an unstable C5 precursor protein or a proteolytic enzyme that destroyed normal pro-C5. Accordingly, intracellular C5 protein produced by and prepared from A/HeJ (C5-deficient) cells was incubated for 30 min at 37 °C with unlabelled, C5-deficient (A/HeJ) conditioned medium, C5-sufficient (Swiss) conditioned medium or fresh medium alone. No degradation of labelled pro-C5 (MW ~ 200,000) or the small MW (38,000) proteins was observed on SDS-polyacrylamide gel electrophoresis of the incubation mixtures.

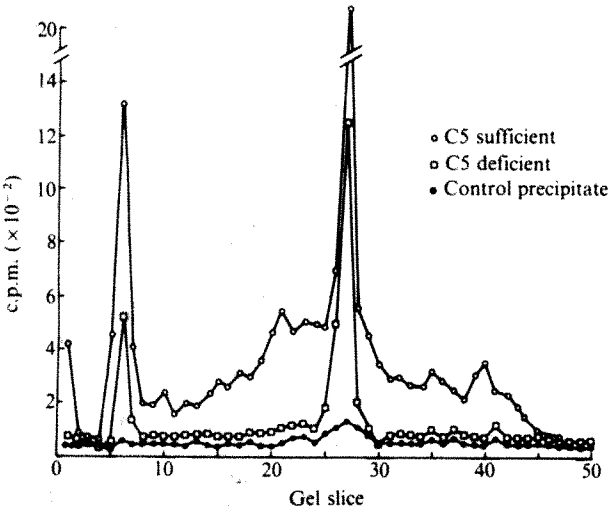


Fig. 2 SDS-polyacrylamide gel electrophoresis of radiolabelled reduced and alkylated C5 protein contained in cell lysates of mouse peritoneal cells from Swiss (C5-sufficient, ○), and A/HeJ (C5-deficient, □) strains. Radioactivity in control immunoprecipitates indicated by closed circles. Estimated MW of the large MW peak was 203,000. In other experiments, estimates ranged from 201,000 to 210,000. The low MW peak was 34,000–38,000. This low MW peak does not correspond in size to a known subunit of C5. A similar pattern of intracellular labelled C5 antigen (two peaks—205,000 and 38,000 MW) was observed in a detergent lysate of Swiss mouse spleen cells obtained from an animal injected with 5 mCi ^{35}S-methionine (supplied by Dr Allen Schwartz).

These data suggest that C5 deficiency in mice is due to a defect in secretion. This may result from a failure of glycosylation as has been proposed as a mechanism for a defect of secretion in certain human immunoglobulin deficiency diseases[8], a mutant mouse myeloma[9] and in normal plasma cells exposed to tunicamycin[10]. A missense mutation could lead to a lack of a suitable site for glycosylation on the C5 polypeptide chain. Alternatively, the defect might reside in synthesis of a protein required specifically for C5 glycosylation or for C5 secretion. In any case, these experiments indicate that the C5 mRNA is translated in cells from several mouse strains genetically deficient in C5, but that the product (pro-C5) is not secreted.

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Bombesin suppresses feeding in rats

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Bombesin (BBS) is a tetradecapeptide originally isolated from amphibian skin[1]. BBS-like immunoactivity is widely distributed in mammalian gut[2–5], and plasma levels have been shown to rise sharply following feeding (ref. 6 and V. Erspamer, personal communication). The physiological actions of BBS are unknown. We have previously shown that the classic gut hormone cholecystokinin (CCK) is a powerful and specific suppressor of food intake[7–9]. Although CCK and BBS lack common amino acid sequences, they have certain common actions on gut viscera[10,11]. We have now shown that BBS also suppresses food intake, and we compare its action with that of CCK.

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Eleven male Sprague–Dawley rats weighing 440–520 g were housed and tested in individual cages on an artificial 12-h light cycle. They were maintained on solid food pellets (Purina) overnight and tested with a balanced liquid food preparation (No. 116EC, GIBCO) diluted 1:3 with deionised water. Solid food was removed at 0930 h each morning, and rats were then allowed access to liquid food for 30 min. This pre-deprivation meal of liquid food assured that all rats had eaten a meal shortly before they were deprived of all food at 1000 h. Immediately before the presentation of liquid food in graduated drinking tubes for the test at 1300 h, rats were injected intraperitoneally (i.p.) with 1 ml of one of the following treatments on different days: (1) synthetic BBS in doses of 2, 4, 6, 8, 16 μg per kg body weight, diluted in 0.15 M NaCl; (2) synthetic C-terminal octapeptide of CCK (CCK8) in doses of 0.2, 0.4, 0.7, 1.4, 2.9 μg per kg, diluted in 0.15 M NaCl; (3) equivolumetric controls of 0.15 M NaCl. Food intake was measured at 5 and 15 min, and then at 15-min intervals for the remainder of the 180-min test. The behaviour of each rat was noted[12] during a 4-s interval every minute for the first 45 min of the test. This systematic observation of the behaviours of each animal permitted calculations of the incidences and pattern of the behaviours associated with feeding (exploration, grooming, apparent sleep), so that control and peptide-injected rats could be quantitatively compared; it also permitted the identification of any abnormal behaviour. In all tests, each animal served as its own control; statistical comparisons were made with a matched-pair *t*-test.

BBS and CCK8 each produced a large suppression of liquid food intake across the entire range of doses tested (Fig. 1). The smallest doses of each peptide produced equivalent 15% suppressions: 2 μg per kg BBS reduced food intake from a control of 18.8 ml to 15.9 ml ($P<0.01$); 0.2 μg (~4 Ivy units) per kg CCK8 reduced intake from 18.6 to 15.8 ml ($P<0.01$). The largest doses of each peptide also produced similar suppressions: 16 μg per kg BBS reduced intake from 20.5 ml to 8.1 ml ($P<0.001$); 2.9 μg (~63 Ivy units) per kg CCK8 reduced intake from 17.1 ml to 5.4 ml ($P<0.001$). A comparison of relative effectiveness on a molar basis revealed that the dose of BBS required to suppress feeding by 50% was five times greater than the equipotent dose of CCK8 (Fig. 1).

In a parallel experiment, the effects of BBS and CCK8 were also tested in animals given solid food (Purina rat pellets) following a 7 h (0900–1600 h) food deprivation. Both peptides reduced solid food intake. In these conditions, the lowest dose of BBS, 3 μg per kg, suppressed feeding by 21% (control intake, 2.8 g; intake after BBS, 2.2 g; $P<0.05$). The lowest dose of CCK8, 1.1 μg (~24 Ivy units) per kg suppressed feeding by 30% (from 2.7 g to 1.9 g; $P<0.01$). The largest dose of BBS tested, 6 μg per kg, suppressed feeding by 50% (from 2.8 g to 1.4 g; $P<0.01$). The largest dose of CCK8, 3.8 μg (~84 Ivy units) per kg, suppressed feeding by 77% (from 2.6 g to 0.6 g; $P<0.001$).

The actions of BBS and CCK8 were similar in two respects. Both peptides suppressed feeding rapidly, with maximum reductions occurring during the first 15 min after injection. In addition, both peptides produced transient suppressive effects: even after large doses, cumulative food intakes of peptide-injected rats had returned to normal levels within 120 min. However, the actions of the two peptides differed in one respect. Although both suppressed liquid and solid food intakes, CCK8 was two to five times as potent in suppressing liquid food intake as it was in suppressing solid food intake, a differential we have noted previously[7]. In contrast, BBS suppressed solid and liquid food intakes equally. Thus, the potency of CCK8 varied according to the physical characteristics of the test diet, whereas the potency of BBS did not.

As BBS suppressed solid as well as liquid food intakes, the suppression was not achieved through some interference with the motor acts required for licking. Furthermore, the largest dose of BBS tested had no effect on water consumption after the minimal water deprivation (6 h) required to achieve reliable intakes. Thus, BBS preferentially reduced feeding, not drinking, even when the same motor act (licking metal spouts for liquid food or water) was required for either ingestive behaviour; this differential strongly suggests that BBS did not reduce feeding simply by producing some generalised discomfort. Several further observations also indicated that BBS did not cause illness or distress. First, the rats never seemed acutely or chronically ill, and they gained weight normally during 8 months of frequent testing. Second, after injections of BBS, rats always began eating eagerly; they merely stopped sooner. Thus, no statistically significant differences in food intake between BBS- and saline-injected rats occurred during the first 5 min of feeding, but BBS-injected rats ate much less than saline-injected rats between 5 and 15 min of the test. This ineffectiveness of BBS in preventing or slowing the early phase of feeding was not an artefact of the time required for the distribution of exogenously injected BBS as it occurred whether the peptide was injected immediately before, 5 min before, or 15 min before food presentation. Thus, BBS preferentially acted to enhance satiety by shortening the meal; it did not prevent or delay the onset of a meal, nor did it slow the initial phase of eating. Third, analysis of the minute-by-minute behavioural data revealed that the only significant behavioural change produced by BBS was a decrease in the incidence of feeding (Table 1).

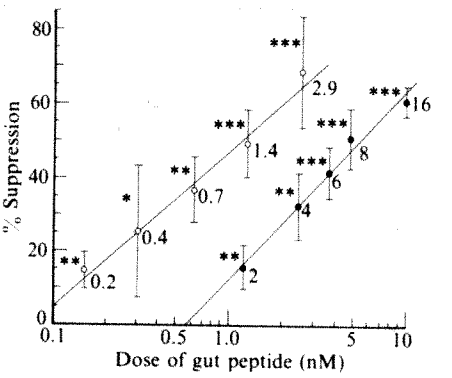


Fig. 1 Suppression of liquid food intake in rats ($n=11$) during the first 15 min after i.p. injections of various doses of BBS (●) or CCK8 (○). The figure shows mean per cent suppressions and s.e.m. compared with intake on control tests. Doses of each peptide are in μg per kg. Doses of the two peptides on the abscissa are arranged along a molar logarithmic scale. Control intakes of liquid food ranged from 13.7 to 20.5 ml (mean 18.1 ml, s.e.m. 0.3 ml). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, statistical differences from respective 0.15 M NaCl controls. Correlation coefficients (Pearson product–moment): for BBS, $r=0.91$ ($P<0.025$); for CCK8, $r=0.97$ ($P<0.005$). Slopes of the regression lines were not significantly different[31] ($t=0.22$, $P>0.05$).

These data demonstrate that BBS did not reduce food intake simply by causing a generalised disruption of behaviour; rather, it produced a selective suppression of feeding, a quality which one would anticipate in a natural satiety signal. Furthermore, in no instance did BBS elicit any abnormal behaviour. Finally, in our unpublished observations, doses of BBS as high as 128 μg per kg have failed to produce any evidence of illness or distress in similar test condictions. Although intracisternal BBS can reduce core body temperature of cold-exposed rats[13], we found no change in core body temperature, as measured by rectal thermoprobe, 15 or 30 min after i.p. injections of doses of 4–32 μg per kg, the times when suppression of feeding was maximal.

The mediation of the satiety action of BBS is unknown. The slopes of the regression lines drawn for the BBS and CCK8 dose–response curves were not significantly different statistically (see Fig. 1). Parallelism of curves is consistent with but not proof of the same mechanism of action[14]. This parallelism might be explained if exogenous BBS produces its satiety effect simply by releasing endogenous CCK[10,15–17]. However, as described above, comparison of the dose–response curves for liquid and solid foods revealed a situation in which the effects of BBS and CCK8 diverged, depending on the physical characteristics of food ingested; this divergence demonstrates that the satiety

Table 1 Incidence of behaviours occurring after i.p. injection of BBS (8 μg per kg) or vehicle control (0.15 M NaCl) in 11 rats

	Feeding	Exploration, grooming	Apparent sleep	Miscellaneous
Control	15	19	65	1
BBS	5*	23	71	1

Incidences are expressed as mean % of the total number of behavioural observations carried out. During the first 45 min of the test the only statistically significant effect which BBS produced was a reduction in the incidence of feeding.

* $P < 0.001$, BBS compared with control. All other BBS–control comparisons were nonsignificant ($P > 0.4$).

action of exogenous BBS cannot be mediated solely by endogenous CCK. Three observations of visceral gut actions of BBS relate to this point. (1) In humans, Basso *et al.*[11] have shown that the addition of secretin to a slow intravenous infusion of BBS caused a partial inhibition, rather than a potentiation, of BBS-induced pancreatic amylase output, suggesting that secondarily released CCK is not the major mediator of BBS-induced amylase output in these conditions. (2) Although enterectomy abolishes BBS-induced pancreatic protein secretion in the dog[15] it does not affect this action in the rat[18]. (3) BBS powerfully stimulates amylase release from *in vitro* preparations of rat pancreatic fragments[19,20] or dispersed acinar cells[21]. These findings strongly suggest that BBS can directly stimulate traditional CCK target tissues.

We conclude that BBS is a putative satiety signal. The existence of at least one unidentified gastric satiety signal has recently been demonstrated in the rat[22,23]. We have shown that the action of this gastric signal does not depend on vagal innervation[24], and may therefore be hormonal in nature. As it is present in large quantities in gastric mucosa[4], BBS is a candidate for this unidentified satiety signal.

We have not determined how the amount of BBS required to elicit satiety in this study relates to the amount and pattern of BBS released by a normal meal, nor how BBS may interact with other putative satiety signals, such as CCK. Finally, the site of action of BBS when it alters satiety behaviour is unknown, but it is of interest that BBS immunoactivity[4,5,25], like that of CCK[26–30], is present in large quantities in the brain.

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Human β-thromboglobulin inhibits PGI_2 production and binds to a specific site in bovine aortic endothelial cells

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Blood platelets have a major role in haemostasis through their aggregation and adherence to the damaged vessel wall. During this process, platelet granular constituents are released. Although the functions of many of these release products are well known, biological functions of some well defined platelet specific proteins have not been determined. This category includes platelet factor 4 (PF4) and β-thromboglobulin (βTG), both small proteins of molecular weight 30,000–36,000 (refs 1, 2) which are stored in the α granule of the platelet and have not been detected in any other tissue. βTG is composed of four identical noncovalently bound subunits and its amino acid sequence is significantly homologous with that of PF4 (ref. 3). Although PF4 possesses a marked ability to neutralise heparin, no physiological or pathophysiological role has been attributed to either protein. The discovery of prostacyclin (PGI_2) has led to a reappraisal of the factors regulating haemostasis and especially of the role of the vascular endothelium in preventing platelet adherence and aggregation[4–6]. The initial action of PGI_2 on the platelet is to stimulate adenylate cyclase[7–9], and it is the most potent inhibitor of platelet aggregation yet discovered. Also, cultures of arterial and venous endothelial cells have been found capable of producing PGI_2 (refs 10, 11). We now report results indicating that βTG reduces the production of PGI_2-like activity in cultured bovine endothelial cells, which are shown to possess a specific receptor for βTG. The characteristics of this receptor suggest that βTG may act locally at high concentrations to favour platelet aggregation by diminishing PGI_2 production.

Monolayer cultures of aortic endothelial cells were established from bovine aortas obtained from animals immediately after slaughter and processed within 1 h. Endothelial cells were prepared from the bovine aorta by collagenase digestion according to the method of Gimbrone *et al.*[12], and grown in plastic culture bottles in medium RPMI-1640 with 40% fetal calf serum. Small groups of cells became established, grew to near confluence within about 1 week and were subcultured by washing the monolayer surface with 0.02% EDTA followed by incubation in 0.025% trypsin–EDTA for 1–2 min. Complete culture medium (5 ml) was then added, and the cells were washed and resuspended in medium to be plated out in fresh vessels. All experiments were carried out on the first to the fourth subcultures. For studies of PGI_2 production, cells were suspended as in routine subculture, but were washed once more before resuspension in a buffered salt solution (BSS). Substances

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to be tested were added to this cell suspension at the desired concentrations, and an aliquot of the endothelial cell suspension containing a known number of cells was tested for its ability to inhibit arachidonic acid-induced aggregation of human blood platelets. Preliminary experiments had shown that incubation of cells with indomethacin (14 μM) or tranylcypromine (500 μM) abolished the ability of endothelial cells to inhibit platelet aggregation. Furthermore, incubation of endothelial cell cultures with ^{3}H- or ^{14}C-labelled arachidonate, followed by extraction and this layer chromatography of acidified medium, showed that the major labelled prostanoid product was 6-keto-prostaglandin $F_{1\alpha}$, which is the stable degradation product of the labile PGI_2. No significant PGE_2 was formed in these conditions of short incubations.

Following incubation with human βTG, significantly more endothelial cells are required for complete inhibition of aggregation than in control incubations (Table 1). PF4, PGE_2, isoprenaline, serotonin and nicotine had no effect in this system. The effect of βTG on endothelial cells was seen at concentrations which are of the same order as those in serum or platelet-rich plasma (PRP) during collagen-induced platelet aggregation[13]. βTG (10 μg ml^{-1}) had no direct effect on the threshold concentration of arachidonate required to induce aggregation, nor did it influence the ability of synthetic PGI_2 to inhibit arachidonate-induced aggregation. These findings indicate that βTG decreases the production of PGI_2-like biological activity by endothelial cells.

Table 1 Effect of incubation with βTG and other substances on endothelial cell numbers required to inhibit platelet aggregation

Treatment	Concentration	Relative no. of endothelial cells to inhibit aggregation		
Control	—	1.00	(23)	
βTG	1 μg ml^{-1}	2.30 ± 0.93	(5)	$P<0.05$
	2 μg ml^{-1}	2.02 ± 0.43	(5)	$P<0.005$
	5 μg ml^{-1}	4.60 ± 0.57	(3)	$P<0.02$
PF4	10 μg ml^{-1}	1.0	(3)	
PGE_2	10^{-6} M	1.0	(1)	
Serotonin	5×10^{-5} M	1.0	(3)	
Isoprenaline	10^{-6} M	1.16 ± 0.05	(3)	NS
Nicotine	10^{-6} M	1.0	(4)	

Results are from 23 individual experiments studying the effects of various agents on the number of endothelial cells required to inhibit arachidonate (AA)-induced aggregation of human blood platelets. Blood was drawn from normal volunteers into 3.8% sodium citrate (1:9 v:v). PRP was prepared by centrifugation at 200*g* for 10 min at room temperature. The threshold concentration of AA (Sigma) required to cause platelet aggregation was determined with 0.4 ml of PRP in a Payton dual-channel aggregometer; normal subjects with no recent aspirin ingestion required 0.4–1 mM AA. Endothelial cell suspensions were prepared by subculturing cells from the primary to the fourth subculture. After the standard subculturing procedure (see text) cells were washed once in phosphate-buffered saline (PBS) and resuspended in BSS of the following composition: Tris 15 mM, NaCl 120 mM, KCl 4 mM, $MgSO_4$ 1.6 mM, NaH_2PO_4 2 mM, $CaCl_2$ 1.2 mM, glucose 10 mM, human serum albumin 0.1%, at *p*H 7.4. Cells were counted in a haemocytometer, and suspended to concentrations of $1–5\times10^6$ per ml. Duplicate incubations of cell suspensions (0.5 ml) were carried out in 10-ml plastic tubes in a shaking incubator at 37 °C. The various treatments were added in BSS, and after at least 15 min incubation, samples of the incubations were tested for their ability to inhibit AA-induced platelet aggregation. βTG was prepared[1,2] from the platelet release products of platelet concentrates and PF4 from the supernatant of outdated platelet concentrates, as previously described[19]. Aggregation inhibition by the incubated cells was tested as follows. To 0.4 ml PRP in an aggregometer tube was added 10 μl of cell suspension or of a dilution made in BSS. Dilutions were made until the minimum amount required to inhibit platelet aggregation was determined, from which the number of cells required to inhibit aggregation was calculated. In each experiment the procedure was repeated for control and treated cells at least twice. Each experiment was completed within 2 h of subculture. In all reported experiments the cell numbers (control and treated) required to inhibit aggregation remained constant throughout the experiment. When this was not the case, experiments were discontinued. The numbers of cells (control incubations) required to abolish threshold AA-induced aggregation varied from 1 to 80×10^3 among the 23 different experiments; in all except 4 experiments a single treatment was tested. Each treatment is expressed as the relative number of cells (±s.d.) required to abolish AA-induced aggregation compared with the control experiment which is expressed as unity; the number of individual experiments is given in parentheses. Statistical analysis was carried out using a logarithmic transformation of the relative cell numbers (paired *t*-test). With PF4, PGE_2, serotonin and nicotine, the effective dilution never differed from that of the control. NS, not significant.

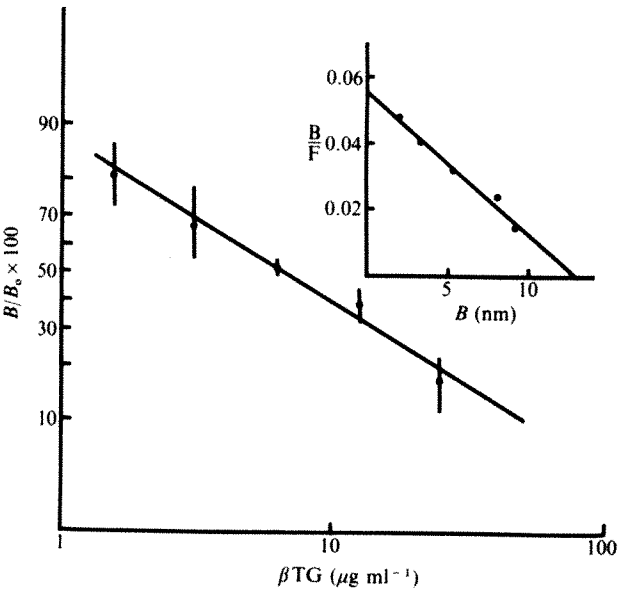


Fig. 1 Specific binding of ^{125}I-βTG to bovine aortic endothelial cells. Cells from the first subculture were dispersed (see text), washed twice with PBS and resuspended at a concentration of 5×10^6 cells ml^{-1} in BSS (see Table 1). Binding experiments were carried out for 1 h at 25 °C in 10-ml plastic tubes with vigorous shaking. Incubations in duplicate consisted of 0.3 ml of cell suspension and 0.2 ml BSS containing ^{125}I-βTG (55,000 c.p.m. per tube) and increasing amounts of unlabelled βTG or other substances to be tested. βTG was labelled with ^{125}I using the lactoperoxidase method as described previously[20]; specific activity in this experiment was 200 μCi per μg, and varied down to 50 μCi per μg in other experiments. At 1 h 200-μl duplicate samples were removed from the incubation, and centrifuged (Beckman Microfuge, Model B, 5 min, 10,000*g*). The supernatants were removed, the tips of the tubes containing the cell pellet cut off and the bound radioactivity estimated in a Packard spectrometer with 75% efficiency for counting ^{125}I. Nonspecific binding (NSB) was measured as radioactivity bound in the presence of excess βTG (50 μg ml^{-1}) and was subtracted from the total radioactivity bound to obtain specific binding. Specific binding of ^{125}I-βTG in the absence of unlabelled βTG (B_0) was 5.1% and the NSB 2.0% in the experiment shown; in other experiments specific binding was between 4 and 9%, and the NSB varied from 0.5 to 2%. The displacement curve is expressed in terms of the ratio of specific binding at a given dose (B) to the specific binding in the absence of added βTG (B_0) plotted on a logistic scale[21]; each point represents the mean of four determinations, and the bars 1 s.d. There was no displacement of ^{125}I-βTG by PF4 (100 μg ml^{-1}), hen egg lysozyme (100 μg ml^{-1}) or human leukaemia lysozyme (100 μg ml^{-1}). Inset: Scatchard plot derived from the displacement curve shown. The dissociation constant (K_d) was estimated as 2.3×10^{-7} (95% confidence limits, 1.7–2.9) and the number of receptors per endothelial cell at 2.6×10^6 (95% confidence limits, $2.1–3.1\times10^6$). B/F, Bound/free βTG.

This effect of βTG suggested that the endothelial cell might possess a cell-surface receptor for βTG. The experiment shown in Fig. 1 indicates the presence of a specific receptor for βTG on bovine aortic endothelial cells. Competition for binding of ^{125}I-labelled βTG was by βTG only and not by PF4 in several-fold excess, nor by other basic proteins, such as hen egg lysozyme (Worthington) or human leukaemic lysozyme[14]. The concentrations of βTG required for half-maximal inhibition of binding are similar to those required to inhibit PGI_2 formation by endothelial cells. In four separate experiments, the average dissociation constant (K_d) was 1.7×10^{-7} M and the number of receptors per cell was calculated as 2×10^6.

Although the methods used to measure the βTG response in endothelial cells do not enable us to relate the effect directly to the binding to endothelial cells, nevertheless, the co-existence of

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a specific binding site and the biological effect strongly suggests that the two are related. Apart from studies of drugs[10], there is little information on other factors which might regulate PGI_2 production by endothelial cells. MacIntyre *et al.*[15] found that a factor in cell-free plasma stimulated PGI_2 production by pig aortic endothelial cells. Thrombin, trypsin and a calcium ionophore have been shown to stimulate PGI_2 production[16], and in experiments similar to our own, plasma low-density lipoproteins have been found to decrease production of PGI_2 activity by human umbilical vein endothelial cells[17]. The present results suggest that βTG may be involved in the control of haemostasis. During vascular wall injury, which is followed by platelet adhesion and aggregation—processes which would both be inhibited by PGI_2—release of βTG could prevent PGI_2 formation by adjacent endothelium, and allow the healing process to continue. In this situation βTG would be acting locally, and the dissociation constant derived from our experiments would be consistent with this action, as concentrations of this order are exceeded during platelet aggregation and release[13]. A local mode of action requires a binding site with an affinity some orders of magnitude less than the normal plasma level of 20 ng ml^{-1} (approximately 0.7 nM)[13]. Bovine endothelial cells were washed free of fetal calf serum before use in the experiments described above. Although bovine βTG has not been reported it is likely that such a protein exists. In this case, although the measured affinity suggests that a significant proportion of bound βTG would dissociate during the washing procedure, the activities measured here may be diminished to some extent by residual occupancy of receptor sites.

It is of particular interest that the distinct but closely related platelet protein, PF4 (ref. 3), neither competes for receptor binding nor has a similar biological effect. βTG has been isolated in two different forms, the preparation used in these experiments corresponding to that originally described by Moore *et al.*[1], its structure has been completely determined[3]. The other preparation, which has been designated low-affinity PF4 (LA-PF4) by Rucinski *et al.*[18], and has an additional NH_2-terminal tetrapeptide but is otherwise identical to βTG, needs to be investigated for probable similar activity. Finally, if βTG interaction with receptor is linked to the effects of the protein on PGI_2 formation, it is important to determine precisely the biochemical events occurring between receptor interaction and PGI_2 production in endothelial cells.

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Inhibition of platelet thrombus formation by chlorpromazine acting to diminish haemolysis

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There is increasing evidence[1] that the sudden, unpredictable event initiating myocardial infarction is fissuring of an atherosclerotic plaque. The resulting haemorrhage into the arterial wall produces obstructive platelet thrombi, just as arterial haemorrhages elsewhere produce haemostatic platelet plugs. It has been suggested[1] that such platelet aggregation depends on ADP originating in red cells which are subjected to excessive haemodynamic stress at the site of haemorrhage. The release of ADP from red cells has been demonstrated *in vitro* in equivalent conditions of shear stress[2,3]; and other mechanisms, such as activation by collagen, cannot account for the rapidity with which the platelets react[4]. One of us (G.V.R.B.) has suggested that drugs capable of counteracting haemolysis[5] might diminish the activating effect of erythrocytes on platelets[6] and so inhibit their aggregation as thrombi. Thus, chlorpromazine, added to human blood at concentrations which diminish haemolysis but do not directly affect platelet aggregation[7,8], prolonged the 'bleeding time' from small holes in artificial vessels where extravasation is terminated, as in living arterioles, by aggregated platelets[9]. The bleeding time was also prolonged by apyrase, consistent with the conclusion that the chlorpromazine acted through decreasing plasma ADP. We show here that this occurs through the anti-haemolytic action of chlorpromazine.

Venous blood was obtained from apparently healthy volunteers who had not taken drugs known to affect platelet reactions. The blood with heparin (20 or 50 units ml^{-1}) as anticoagulant was pumped at 37 °C from a syringe through polyethylene tubing of 280 μm internal diameter and 50 cm long. The flow rate was 0.17 or 0.21 ml min^{-1} (both rates gave the same results) and the pressure was about 300 mm Hg. The duration of 'bleeding' from a small cut through about one half of the circumference of the tube and 30 cm from its end was determined up to 600 s. Free haemoglobin was determined by the cyan-methaemoglobin method in blood from the syringe ('unsheared') and from the end of the tube ('sheared'). Most determinations on unsheared blood were bracketed between two on sheared blood.

It was confirmed[9] that chlorpromazine added to the syringe blood for 45 min prolonged the bleeding time significantly, and did so more at 5 or 10 μM than at 50 μM (Table 1).

Unsheared blood contained low concentrations of free haemoglobin which were not changed significantly by chlorpromazine at 5 or 10 μM but were increased by drug concentrations of 50 μM (Table 1). In sheared blood free haemoglobin concentrations were significantly higher than in unsheared blood in the absence of chlorpromazine, which decreased the free haemoglobin significantly at 5 μM and just significantly at 10 μM but not at 50 μM. The differences in free haemoglobin between unsheared and sheared blood were decreased highly significantly by chlorpromazine but less at 50 μM than at 5 or 10 μM.

Our interpretation that the prolongation of bleeding time by chlorpromazine depends on its effect on red cells is tenable only if direct inhibition of platelet aggregation could be excluded. In three experiments it was confirmed (see ref. 8) that chlorpromazine incubated at these concentrations in blood or pla-

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Table 1 Effect of chlorpromazine on bleeding times and haemolysis of heparinised blood during its shear-flow through artificial vessels

No. of determinations		Chlorpromazine concentration	Bleeding time (s)	Free haemoglobin (mg per 100 ml) in blood		
Unsheared	Sheared	(μM)	mean s.e.m.	Unsheared mean s.e.m.	Sheared mean s.e.m.	Difference mean s.e.m.
15	15	0 (Controls)	178 ± 23	33.4 ± 1.4	44.6 ± 2.2	11.2 ± 1.9
14	14	5	242 ± 28*	32.3 ± 3.5	35.9 ± 3.5	3.6 ± 0.9†
14	14	10	288 ± 32*	37.2 ± 2.8	38.1 ± 3.3	0.9 ± 1.1†
15	15	50	263 ± 30*	41.3 ± 3.8	44.7 ± 3.9	3.4 ± 2.1†

Significances of differences from controls: *$P < 0.05$; †$P < 0.005$.

telet-rich plasma for increasing lengths of time had no significant effect on the velocity of primary aggregation[10] induced by ADP. Secondary aggregation associated with the release reaction is not inhibited by chlorpromazine at 5 μM, slightly at 10 μM and more at 50 μM (ref. 8), whereas the bleeding time was more prolonged and haemolysis more strongly inhibited at 5 μM and 10 μM than at 50 μM.

These observations establish that low concentrations of chlorpromazine greatly diminish haemolysis of blood during shear-flow but not when at rest. This strongly supports the proposition[9] that chlorpromazine increases the bleeding time through its anti-haemolytic effect. In the absence of chlorpromazine, haemolysis would have given initial plasma concentrations of ADP and ATP of 0.05 and 0.40 μM respectively. Because the dephosphorylation in blood of ATP to ADP is much faster than that of ADP to AMP[11], the concentration of ADP was probably higher and amply sufficient to activate platelets[12]. It can be concluded, therefore, that ADP released from red cells lysed by haemorrhagic shear-flow can account for haemostatic and thrombotic platelet aggregation.

The haemolysis caused by shear-flow was strikingly decreased by chlorpromazine. On the assumption that these decreases in the release of haemoglobin were accompanied by corresponding decreases in the release of ADP and ATP, it may be concluded that the increases in bleeding time brought about by these concentrations of chlorpromazine are explained by the very much lower concentrations of ADP available for activating the platelets.

This explanation is supported by the apparently paradoxical diminutions in the effect on both bleeding time and haemolysis when chlorpromazine was present at the higher concentration of 50 μM. Chlorpromazine, like other drugs (see ref. 5), is anti-haemolytic at low but strongly haemolytic at higher concentrations. It seems, therefore, that in our experiments chlorpromazine at 10 μM was almost maximally anti-haemolytic whereas at 50 μM the opposing haemolytic effect began to show itself also.

While the blood was in the syringe reservoirs at 37 °C it haemolysed to a small extent (0.23 ± 0.02% in blood from 8 donors) after 2–3 h. In such a long time the adenine nucleotides released from lysed red cells are enzymatically dephosphorylated[11] so that there would be no free ADP to activate the platelets in the blood reservoirs.

Red cells undergo the greatest haemodynamic stress near the vessel walls, where flow velocity is slowest. Therefore, the concentration of released ADP is greatest near the walls which is where the platelets have to be activated for their haemostatic function.

The wall shear stress in these artificial vessels was about 50 dyn cm^{-2}, which is similar to that in living arterioles[13]. This stress, even when acting for considerably more than 10 s, is too small to activate platelets directly[14]. The wall shear stress is higher by at least two orders of magnitude at sites of arterial haemorrhage[3]. This stress is too brief to activate platelets directly[14]. Their activation can now be accounted for by assuming the local lysis of a very small proportion of red cells.

There is evidence[15,16] which can be interpreted[17] to suggest that chlorpromazine and at least one other phenothiazine administered for clinical purposes are capable of protecting against myocardial infarction. As the concentration of chlorpromazine in patients' plasma[18] is about one order of magnitude lower than that which greatly prolonged the *ex vivo* bleeding time, blood concentrations of chlorpromazine produced clinically may protect against myocardial infarction by the anti-haemolytic mechanism.

All this evidence supports the suggestion[1,9,17] that drugs which have the effect described in this paper for chlorpromazine may be found to ameliorate or prevent arterial, for example coronary, thrombosis when it is induced by haemorrhage into atheromatous lesions or by other haemodynamic stresses severe enough to cause haemolysis.

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Electron microscope evidence for an 80 Å unit in collagen fibrils

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Connective tissues are composite structures containing collagen, elastin, glycosaminoglycans, minerals, water and other minor components[1,2]. In all cases the collagen exists predominantly in fibrillar form. The size distribution of the fibrils does, however, vary markedly with both age and the mechanical requirements of the tissue[3]. Little is known about the mechanism of fibril formation *in vivo*, although some information is now available from *in vitro* polymerisation studies[4]. We have now collected new and extensive electron microscope data on the size of collagen fibrils from tendon, skin, cornea and other tissues from both fetal and immature animals. The results reported here show that the diameters of the collagen fibrils thus measured lie close to a multiple of 80 Å, a result which may be simply and readily interpreted in terms of the collagen microfibril and its mode of packing.

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Table 1 Classification of the mean diameters of collagen fibrils having sharp unimodal populations

Tissue	Species	Age*	Collagen fibril diameter n.80 Å				
			$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
Tendon: Triceps	Rat	0 day	160				
Triceps	Rat	0 day		250			
Tail	Rat	18 day F		230			
Tail	Rat	0 day			305		
Tail	Rat	<1 day					485
Upper forelimb	Rat	18 day F			320		
Forelimb digital flexor	Rat	18 day F		230			
Forelimb digital flexor	Rat	0 day			310		
Hindlimb digital flexor	Rat	18 day F	175				
Hindlimb digital flexor	Rat	5 day				405	
Forelimb	Sheep	60 day F		245			
Forelimb	Sheep	60 day F			305		
Hindlimb	Sheep	60 day F			330		
Hindlimb digital flexor	Sheep	72 day F				395	
Hindlimb digital extensor	Sheep	72 day F					490
Cornea (a) Cartilaginous fish	Dogfish	A		245			
	Elephant fish	A		245			
	Stingray	A		235			
(b) Bony fish	Butterfish	A	160				
	Moki	A	165				
(c) Amphibians	Salamander	A		235			
	Toad	A		230			
(d) Reptiles	Turtle	A		235			
(e) Birds	Kiwi	A		245			
(f) Mammals	Opossum	A		245			
	Rat	18 day F	175				
	Rat	0 day		225			
	Rat	A		235			
	Rabbit	A		250			
	Human	14 and 24 week F	170				
	Human	73 year		225			
Skin	Rat	18 day F		240			
	Rat	0 day			320		
	Sheep	60 and 72 day F			300		
	Human	14 week F		250			
Endoneurium: Sciatic nerve	Rat	0 day		240			
Sciatic nerve	Rat	5 day			310		
Paratenon: Tail tendon	Rat	0 day		245			

* A, Adult: F, fetal.

It is generally accepted that collagen fibrils are constructed from molecules which are axially staggered with respect to one another by about 668 Å (or a multiple thereof)[5–9]. The nature of the lateral packing of the molecules is controversial, however. The first significant packing model proposed was that of Smith[10], who suggested that a five-stranded microfibril of diameter ~35 Å could exist in connective tissues. Improved X-ray data from orientated specimens of moist rat-tail tendon subsequently became available and these confirmed that a unit of diameter ~38 Å was indeed present[11], thus providing strong support for the Smith microfibril. Further interpretation of the X-ray patterns[12–14] showed that the unit cell was a slightly sheared tetragonal cell of lateral side ~77 Å and that each unit cell contained four microfibrils related to one another by a 4_3 screw axis. Others have since suggested alternative schemes for the packing of collagen molecules in the fibril (see for example refs 15–17) and in each instance it is claimed that the X-ray data are consistent with the proposed scheme. Electron microscope evidence has therefore been sought to differentiate between the various models which have been put forward. At the present time, however, no conclusive evidence has been obtained from transverse sections which supports a regular arrangement of collagen molecules or groupings of collagen molecules though the possibility remains that the preparative procedures used have not preserved the *in vivo* lattice structure.

It has been suggested by Fraser *et al.*[18] that if the structure of small collagen fibrils, which appear circular in section, is indeed based on a lateral repeating unit of four microfibrils related to one another by a 4_3 screw axis, then their diameters should be multiples of about 85 Å. This value is calculated on the basis of the most symmetrical arrangement of lateral repeating units, that is, an $n \times n$ array. Fraser *et al.*[18] noted that the preservation of the structure of small fibrils would probably be insufficient to distinguish between $n \times n$ unit cell fibrils and helical fibrils, both of which would appear to be circular in cross-section. Parry *et al.*[19] simultaneously reported that the mean diameter of the collagen fibrils from 10 tissues, each of which had a sharp unimodal distribution of fibril diameters, occurred at multiples of about 80 Å. We present new data which support those preliminary observations and which imply that the unit cell and microfibrillar structure proposed for tendon are likely to be a feature of all other connective tissues as well.

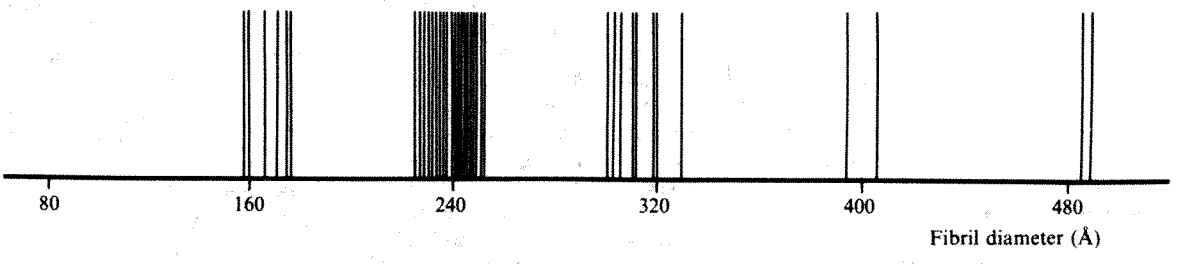


Fig. 1 Graphical representation of the values listed in Table 1. It can be seen that the diameters of the collagen fibrils fall in discrete clusters separated from one another by 80 Å. The clusters are centred on values of about 160, 240, 320, 400 and 480 Å.

Let us consider the conditions in which quantum jumps of 80 Å in fibril diameter might most easily be observed. Firstly, the magnification of the electron microscope must be known precisely for each set of micrographs taken, the calibration being based, say, on a replica grating. Secondly, the mean value of many diameter measurements ($\geq$100) taken from the original electron micrographs using a binocular microscope, will only be close to the true diameter of each and every fibril (and hence be meaningful) if the distribution of collagen fibril diameters is both unimodal and sharp. This assumes that the spread of measured values does not represent the true range of collagen fibril diameters but arises entirely from the inherent difficulty of measuring and defining the fibril periphery after standard electron microscopic preparative procedures have been used.

The standard deviation for each distribution of collagen fibril diameters has been calculated and where this is <35 Å it has been considered that the distribution is sufficiently sharp to represent a population of uniform diameter fibrils. The stipulated standard deviation, being less than (80/2) Å, gives a criterion on which to exclude those fibril diameter distributions which are likely to be comprised of two or more populations of fibrils differing in diameter from one another by a multiple of 80 Å. Such distributions will have a mean diameter dependent solely on the relative proportions of the various populations of fibrils making up the overall distribution.

Measurements made in this work, together with those of Parry *et al.*[19], are shown in Table 1 and Fig. 1. The results show that the diameters of small collagen fibrils taken from a variety of diverse connective tissues have values which are close to a multiple of about 80 Å. It is important to note that Table 1 contains every piece of data that we have collected, subject only to it satisfying the stated criteria. We are aware of many values quoted in the literature which relate to the diameters of small collagen fibrils and while many of these values fall close to a multiple of 80 Å (see for example ref. 20), others do not. We believe that it is probable that most, if not all, of these values do not satisfy one or both of the criteria listed earlier.

It therefore seems appropriate to suggest that the 80 Å unit cell first seen in tendon from X-ray diffraction analyses and now shown by electron microscopy to exist also in tissues such as skin, cornea and endoneurium, as well as tendon, may have a similar interpretation in all cases, that is, each 80 Å unit contains four microfibrils related to one another by a 4_3 screw axis.

We believe that the diameters of all collagen fibrils, irrespective of their size, may be multiples of about 80 Å. This cannot, of course, be easily proved in the cases of broad distributions of collagen fibrils as a meaningful mean diameter (within the limits imposed by our criteria) can no longer be calculated and also because any slight ellipticity of a single fibril in cross-section will disguise its true diameter. Also the shape of the collagen fibrils in cross-section often become less regular as the fibrils increase in diameter and as the tissue ages[21,22]. Nonetheless we propose, on the basis of these results, that all fibril diameter distributions, whether they originate from a fetal, mature or senescent tissue, are constructed from changing populations of collagen fibrils whose diameters are multiples of about 80 Å.

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Competence for genetic transformation in pneumococcus depends on synthesis of a small set of proteins

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In bacterial genetic transformation the uptake of DNA and its integration into the resident chromosome is dependent on a special cellular state, termed competence. In those species where appearance of competence has been studied, specific (but often poorly defined) growth conditions lead to a simultaneous development of competence in a substantial fraction of the cells in a culture. In *Bacillus subtilis*[1–4], and in *Haemophilus* species[5,6], competence appears in the stationary phase of growth or in certain other growth-limiting conditions[5,6]. *Streptococcus pneumoniae* (pneumococcus) is perhaps unusual in that virtually all cells of a culture become competent[7,8], for a short period at a specific cell density during logarithmic growth, without perturbing the growth rate[9]. The synchronous appearance of competence in pneumococcal cultures results from an autocatalytic effect of a small protein[10] released by the cells that induces competence[11,12]. The response to competence factor has been shown to require protein synthesis[13]. We report here additional information on the nature of competence in pneumococcus: pulse-labelling studies show that for the brief period of competence protein synthesis is restricted to a few specific polypeptides.

Figure 1 displays the patterns of proteins pulse-labelled with ^{35}S-methionine at successive intervals before, during, and after competence in a culture transferred to a low-methionine labelling medium after stimulation by endogenous competence factor. Also shown is the level of competence achieved by the end of each labelling pulse, measured genetically. The fluorogram reveals two prominent features of protein synthesis associated with the appearance of competence. First, there is a striking decrease in the synthesis of most proteins, including all the major labelled bands. Second, during the middle portion of the competence period, protein synthesis, although normal in total amount, is limited to about 11 principal species. The approximate molecular weights of these are: 14.5K, 16K, 18K, 19.5K, 31K, 34K, 36K, 41K, 45K, 50K, and 52K. Only one of these, about 19,500 MW, is clearly absent from the pattern of non-competent cells at this level of resolution. Critical comparison of lanes in the original film suggests that at least three others are new, but a second dimension will be needed to verify this. Thereafter, as competence declines, the labelling of these bands decreases, and synthesis of the normal complement of proteins resumes.

In a second experiment, the synthesis of the same set of proteins appeared in samples of a culture that became competent, but not in samples of a parallel culture grown with trypsin to inactivate the intercellular action of competence factor (Fig. 2).

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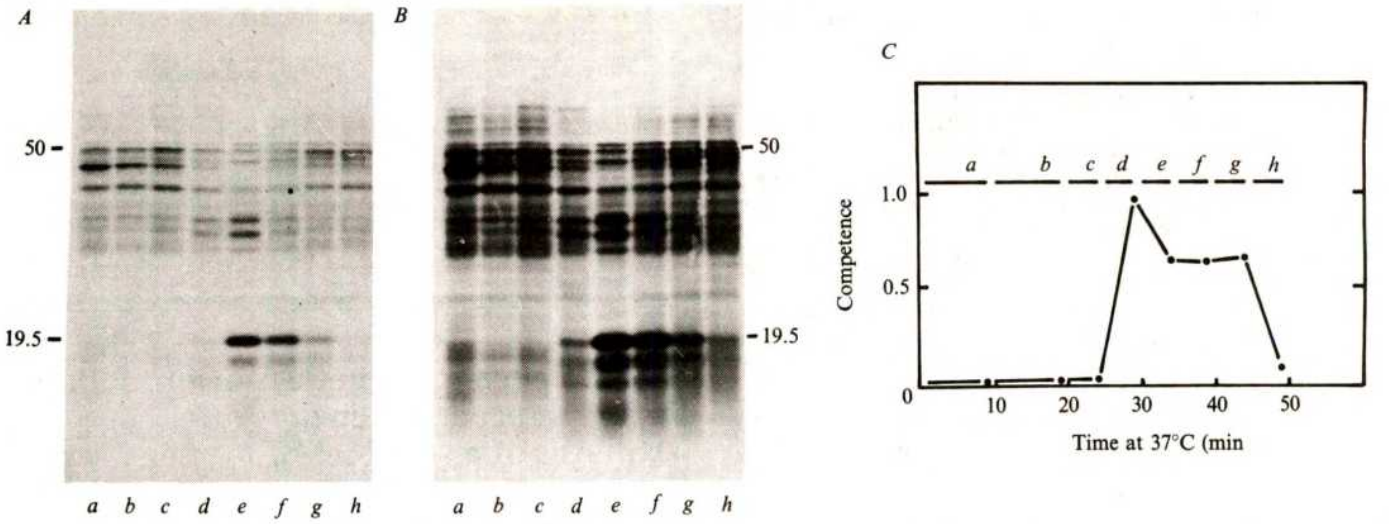


Fig. 1 Pulse-labelled proteins of a pneumococcal culture developing competence for genetic transformation. A growing culture of DP1700 was chilled about 25 min before the peak of competence, concentrated to 4×10^8 colony-forming units per ml and frozen[14]. A sample was thawed at 0 °C, washed and resuspended in an equal volume of labelling medium[16]. During incubation of this sample at 37 °C, aliquots were labelled with ^{35}S-methionine (12 μCi ml^{-1}) for periods, shown by bars in panel *C*, then assayed for competence by dilution of a portion into broth containing 2 μg ml^{-1} Ery-r transforming DNA, and prepared for electrophoresis. Cells were chilled, washed, and resuspended in 10 mM Tris, *p*H 6.8, 1 mM EDTA, 5 mM $MgCl_2$, 0.1% Triton X-100, 10 μg ml^{-1} DNase I, 50 μg ml^{-1} RNase, and lysed at 37 °C (10 min). Lysates, boiled with 1% mercaptoethanol and SDS, were applied to a 12% slab gel. The $0.15 \times 10 \times 14$ cm polyacrylamide gel used the SDS disc buffer system of Laemmli[17], and fluorography was as described by Laskey and Mills[18], with Kodak Xomat XR-1 film. Each well of the slab gel contained 10^4 c.p.m. incorporated ^{35}S. *A*, Fluorogram of samples labelled for the periods indicated in *C*, 6-h exposure. *B*, Same as *A* at 18-h exposure. *C*, Course of competence development. Maximum competence represents 10^6 Ery-r transformants per ml per μg DNA, after a 7-min uptake period at 25 °C. Molecular weights were determined from positions of reference proteins listed in text. (Strain DP1700 is a derivative of Rx-1 carrying the *end-1 exo-2* mutations isolated in R_6 by Lacks[19], and was supplied by N. S. Shoemaker. In our hands it exhibits a tendency to lyse if handled soon after competence[11,12], seen here as low recovery of ^{35}S in sample *d* of Fig. 2. Such lysis did not occur in any of the samples of Fig. 1, as shown by complete recovery of incorporated radioactivity, and by Coumassie blue staining after electrophoresis.)

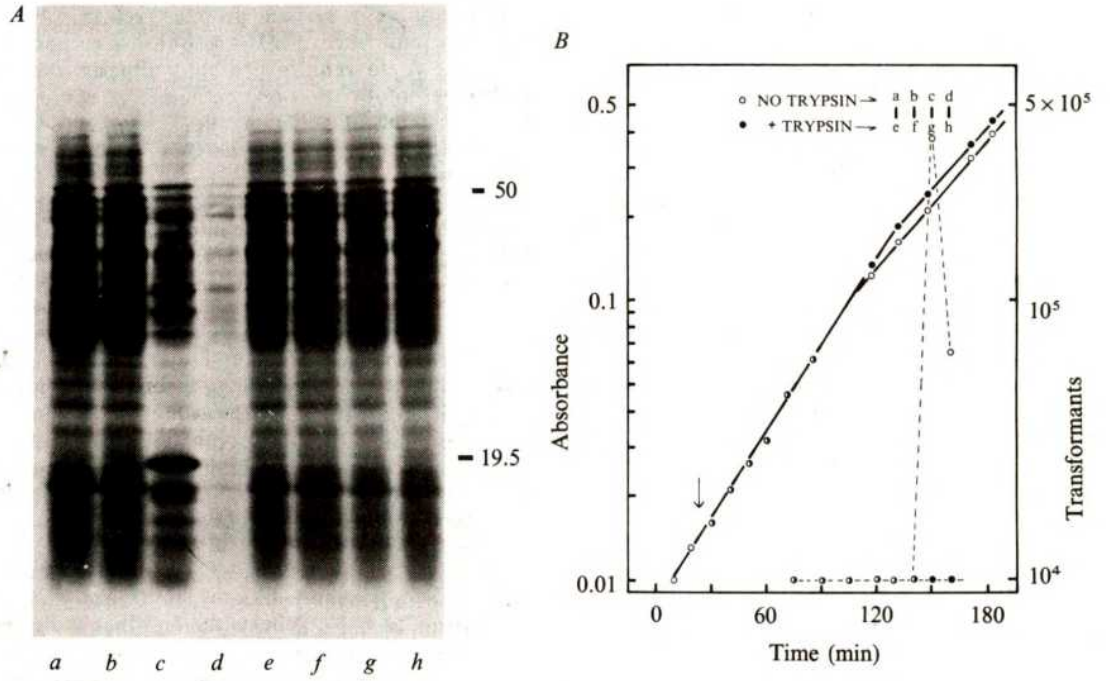


Fig. 2 Effect of trypsin on competence development and on protein synthesis patterns. A culture of DP1700 growing at 37 °C in competence medium[14] was split at the time indicated by the arrow and one half was supplemented with trypsin to 20 μg ml^{-1} (gel lanes *e–h*). The other half was an untreated control (gel lanes *a–d*). Growth was monitored at 430 nm in the Coleman jr II spectrophotometer. At intervals, samples were removed, chilled, frozen, thawed, transferred to labelling medium containing 2 μCi ml^{-1} ^{35}S-methionine, and incubated at 37 °C for 20 min. A portion of each sample was then assayed for competence, and the rest prepared for electrophoresis and analysed, as in Fig. 1. Panel *A*, fluorogram of gel for samples taken at the times indicated in panel *B*, *a–d* control, *e–h* trypsin treated. Panel *B*, culture growth and competence assays. ●, Trypsin treated; ○, control; ———, optical density of culture; - - - - - - -, competence.

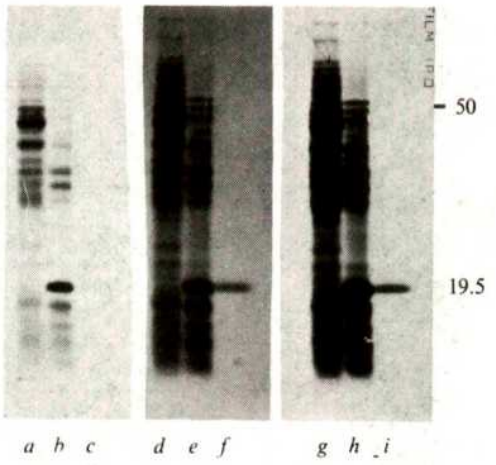


Fig. 3 Comparison of pulse-labelled proteins with purified eclipse complex protein. A lysate of component cells (*b*) labelled as in Fig. 1 (pulse *e*), was analysed on a slab gel as in Fig. 1. Adjacent lanes contained: *c*, protein obtained from ^{35}S-methionine-labelled eclipse complex purified as described elsewhere[14]; and *a*, a lysate of pulse-labelled non-competent cells. Three fluorography exposures are shown: *a–c*, 6 h; *d–f*, 12 h; and *g–i*, 18 h.

We suggest that this event, coinciding precisely with the appearance of competence, may represent the coordinated synthesis of sufficient amounts of the proteins involved in uptake and/or recombination of DNA to accomplish the rapid uptake and integration of large amounts of DNA.

Figure 3 compares the proteins labelled specifically at competence with the protein which coats donor DNA in the competent cell during eclipse, after uptake and before integration into the chromosome[14,15]. The principal competence-specific band co-migrated precisely with pure eclipse complex protein, and may consist wholly of that DNA-binding protein. By comparison with standards of BSA (MW 66,000), trypsinogen (MW 25,000), β-lactoglobulin (MW 18,400) and lysozyme (MW 14,000), we estimate the MW of this polypeptide to be $19.5 \pm 0.5 \times 10^3$, a value somewhat larger than that estimated previously[15] in an analysis heavily dependent on the mobility of cytochrome *c*.

The competence-specific 19,500 MW protein and the 19,500 MW protein of eclipse complex have not yet been directly shown to be identical; however, the correlation with competence of synthesis of the former, the binding of the latter to transforming DNA in the cell, and their identical electrophoretic mobilities strongly suggest that they will ultimately be found so.

Evidence on the nature of the competent state in any species is limited. One extreme hypothesis is that competence reflects simply the acquisition of a transport system for DNA, with constitutive proteins of nucleic acid metabolism carrying out the remaining steps. On this view, the mechanism of integration could simply represent the ordinary fate of free homologous intracellular strands of DNA. At another extreme is the hypothesis that during ordinary nucleic acid metabolism the reactions required for genetic transformation are unusual, and that competence, then, requires the coordinated synthesis of specific proteins not usually present in sufficient quantity to carry out these reactions. The elaborate shift to the synthesis of few proteins that we report here accompanies competence certainly suggests the possibility of the latter case.

Clearly, specific assignments of roles in transformation to these apparently competence-specific proteins will depend on further research involving a combination of genetic and biochemical approaches. The major competence-specific protein, p19.5, of course, is already apparently implicated by its binding to DNA in eclipse. If even a few others should also be identified, then pneumococcal competence would constitute a particularly clear example of an inducible bacterial system for genetic recombination.

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Renal conservation of antifreeze peptide in Antarctic eelpout, *Rhigophila dearborni*

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In Antarctic notothenioid fishes large amounts (3% w/v) of small molecular weight glycoproteins lower the freezing point of the body fluids a few tenths of a degree below the freezing point of seawater (−1.9 °C), enabling these animals to avoid freezing[1]. Although these glycoproteins have molecular weights of 2,600–23,500 and would be expected to be filtered into the urine, they remain in the blood because the kidneys of these fishes contain only aglomerular nephrons[2,3]. Unlike the situation in most fishes, urine formation is the result of secretion rather than filtration and reabsorption. On the other hand, the peptide antifreezes in Northern Hemisphere fishes such as the winter flounder[4], *Pseudopleuronectes americanus*, are retained by the glomerular kidney even though inulin, of comparable weight, is rapidly filtered from the blood into the urine[5]. The Antarctic eelpout (zoarcid), *Rhigophila dearborni*, which is unrelated to either the Antarctic notothenioids or *P. americanus*, also uses a peptide antifreeze (molecular weight 6,000) which is maintained at a concentration of 3% (w/v) in the blood plasma. We report here that the lack of antifreeze in the urine of *R. dearborni* probably reflects the fact that the glomeruli are not functional and cannot filter. We support this conclusion with morphological and physiological evidence and relate our findings to the conservation of biological antifreeze necessary for life in ice-laden polar waters.

Rhigophila were collected in baited traps lowered to a depth of 475–550 m through the ice at McMurdo Sound, Antarctica (77° 54′ S, 166° 40′ E). The traps were retrieved 4 d later with an oceanographic winch. Fish were maintained in aquaria supplied with running water from McMurdo Sound.

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Although *Rhigophila* has been reported to have glomerular kidneys[3], we could not detect any peptide antifreeze in its urine on the basis of the difference between the melting point and the freezing point. We assessed glomerular filtration rate by infusing the caudal vein with the low molecular weight polysaccharide ^{14}C-inulin which is neither secreted nor reabsorbed by the tubular epithelium. In 100 h little inulin appeared in the bladder urine. The mean ratio of ^{14}C-inulin in urine and plasma was 0.017 (5 fish), similar to values reported for the aglomerular Antarctic cods, *Dissostichus mawsoni* and *Trematomus bernacchii*[2]. During infusion, urine flow in *Rhigophila* averaged 0.285 ml h^{-1} kg^{-1} of wet weight. When ^{3}H-*p*-aminohippuric acid was infused, it appeared in the bladder urine within 1 h and maximum concentrations were observed after about 3 h when the urine/plasma ratio averaged 230. These results indicated that filtration was not involved in urine formation in *Rhigophila* even though the rate of production was similar to that of other glomerular marine teleosts.

These physiological findings led us to investigate the structure of the seemingly non-functional glomeruli of *Rhigophila*. Kidney samples were fixed for histology and electron microscopy by conventional techniques. Most Nephrons were aglomerular, and *Rhigophila* should be considered a pauciglomerular fish. The glomeruli were small compared to tubule diameter. Although some glomeruli had a few patent capillary loops, most exhibited no vascularity. Nephrons with glomeruli had short neck segments. The only other recognisable regions of the nephron were the second proximal (principal) segment and the collecting tubule, resembling the situation in truly aglomerular fishes[6].

The squamous epithelium of the parietal layer of Bowman's capsule continued without change into the very short neck portion of the nephron. Although the lumina of Bowman's space and the neck were easily seen, it was difficult to demonstrate a direct connection between the lumina of the neck and the rest of the nephron. Serial section reconstruction verified that the epithelial components of the two portions were continuous, but continuity of the lumina could not be established. If significant amounts of glomerular filtrate were produced, the passage of filtrate into the neck would be expected to keep the lumen visible. The paucity of vascular loops within the glomerulus and the failure to demonstrate luminal patency in a portion of the nephron suggested that there was little or no filtration.

As a further test of glomerular vessel patency, Microfil (Canton Bio-Medical Products) was gravity perfused through the ventral aorta after blood had been washed out with heparinised marine teleost Ringer solution. Small arterial vessels on the capsule and in the interior of the kidney were filled, but we found no evidence of the Microfil in glomerular capillaries. We concluded that the blood supply to the kidney of *Rhigophila* was predominantly venous, as in aglomerular fishes[6].

Ultrastructural studies confirmed that the glomeruli of *Rhigophila* were not functional. The endothelium of the glomerular capillaries was thick and lacked fenestrae (Fig. 1). The basement membranes were also thick (Fig. 1), presumably from the deposition of material produced by the mesangial cells. The cells of the visceral layer of Bowman's capsule lacked the characteristic podocyte appearance including pedicles and filtration slits (Fig. 1). These features resemble the glomerular characteristics associated with certain mammalian kidney diseases[6,7]. We do not interpret the glomerular morphology of *Rhigophila* as pathological, but rather as a consequence of degeneration during evolution. A similar situation occurs during the regression of the larval opisthonephros in the sea lamprey, *Petromyzon marinus*[7].

Ultrastructural features of the cells forming the principal part of the nephron included a brush border with occasional cilia, apical tubules and vesicles, numerous junctional complexes and smooth muscle cells around the tubule. There were very few lysosomes in the apical cytoplasm of the tubule cells. These organelles are normally present in tubule cells active in filtrate reabsorption[6]. Their scarcity was another indication of reduced glomerular filtration in *Rhigophila*.

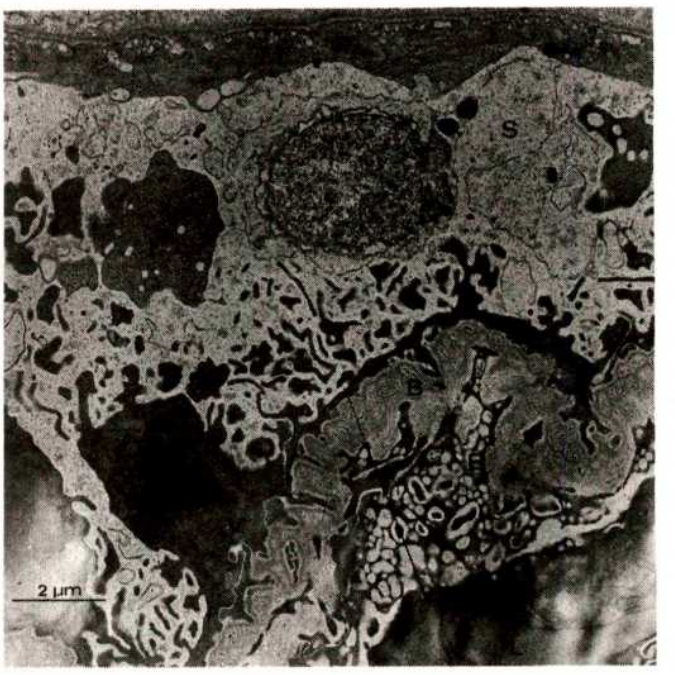


Fig. 1 Electron micrograph of a renal corpuscle from *Rhigophila*. P, parietal layer of Bowman's capsule; V, visceral layer (podocytes) of Bowman's capsule; S, Bowman's space; B, basement membrane; E, endothelium and L, lumen of glomerular capillary. The glomerular endothelium lacks fenestrations and the podocytes lack pedicles and filtration slits. Double-headed arrow indicates the thickness of the filtration barrier (about 5.0 μm).

Rhigophila antifreeze peptide molecules are negatively charged (isoelectric point 5.5–6.0). Research on mammalian kidneys has shown that the passage of negatively charged molecules through the filtration barrier is restricted by carboxyl-rich glycoproteins in the subendothelial and subepithelial layers of the glomerular basement membrane[8–11]. Although this mechanism may not be important in the thick glomerular walls of *Rhigophila*, it may be operational in the related north Atlantic eelpout, *Macrozoarces americanus*, and in *P. americanus*. These species filter inulin freely and also possess acidic peptides with antifreeze properties during the winter (A.L.D., unpublished data).

The reduction or loss of glomerular surface area in marine teleosts has been viewed as an energy conserving mechanism[6]. By reducing the number of small organic solutes and neutral macromolecules escaping from the plasma at the glomerulus, energy need not be expended in reabsorbing these 'useful' components from the filtrate[6]. The pauci- and aglomerular kidneys of many polar fishes have the additional advantage of preventing or minimising urinary loss of small peptides and glycoproteins with antifreeze properties[2]. Energy conservation is likely to have been especially important in the adaptation of polar fishes to their environment, and the unusual kidneys of *Rhigophila* are an intriguing manifestation of this adaptation.

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APPOINTMENTS VACANT

UNIVERSITY OF THE WITWATERSRAND
Johannesburg
DEPARTMENT OF ANATOMY
SENIOR LECTURERS/ LECTURERS

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin, for appointment to the above two posts, which are available from January 1980. One of these posts is a contract appointment for one year in the first instance. Candidates should have an interest in one or more of the following branches of Anatomy: Human Gross Anatomy, Neuro-anatomy, Comparative Morphology and any of the subdivisions of Human Biology.

The salary attached to these posts is within the following ranges: Senior Lecturer: R15 000 — R19 500 per annum. Lecturer: R8 100 — R16 800 per annum (£1 = R1.77 approx).

Applicants not meeting the required standard of qualifications and/or experience may be offered appointment at a lower level.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged by 30th November 1979. 3704(A)

STANFORD UNIVERSITY
GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

METAMORPHIC PETROLOGIST
DEPARTMENT OF GEOLOGICAL SCIENCES
THE UNIVERSITY OF TEXAS AT AUSTIN

A tenure-track faculty position is now open in metamorphic petrology, effective September 1980. The candidates must be field oriented and able to use modern laboratory instruments for research and teaching. An interest in mineral resources is desirable. Teaching responsibilities will include graduate and undergraduate metamorphic petrology, undergraduate mineralogy and field instruction, and supervision of graduate research.

Applicants for this position must have completed all requirements for the Ph.D. degree at the time of employment. Please send complete résumé, including names of three references, an abstract of the dissertation, statement of research interests and planned research projects to: Dr Robert E Boyer, Chairman, Department of Geological Sciences, The University of Texas at Austin, P.O. Box 7909, Austin, Texas 78712.

Deadline for applications: January 15, 1980. Equal Opportunity/Affirmative Action Employer, M/F. W519(A)

ATHROFA GOGLEDD-DD CYMRU
The North East Wales Institute of Higher Education
SCHOOL OF NATURAL SCIENCE (RESEARCH DIVISION)
Kelsterton College
RESEARCH ASSISTANTS

Two posts are available, initially for a 2 year period, for a suitably qualified:
1. Biochemist, with interests in cellular and intermediary metabolism.
2. Cell biologist, preferably with experience in tissue culture techniques.

Both posts are industrially-sponsored, and form part of the Institute's programme of drug research, under the general supervision of Dr J C Allen, the Head of the Research Division. The Division possesses well equipped animal and tissue culture facilities.

Appointment will be on the T3/4 Grade of the NJC scales, with salary between £4,080 and £5,067 pa. Applicants, who should be Honours Graduates, and who will have the opportunity to register for a higher degree, should apply to The Institute Registrar, Kelsterton College, Connah's Quay, Clwyd, before 30th November, 1979. 3695(A)

CELL BIOLOGIST
(Associate Professor)

Applications are solicited for a nine-month tenure track faculty position in cell biology. All qualified cell biologists (animal and plant) without regard to specialty are encouraged to apply. The appointment will be at the Associate Professor level and will be available Autumn, 1980. Applicants must have demonstrated excellence in research and teaching. Responsibilities of the successful candidate will include developing and directing a quality research program in some aspect of cell biology, advising graduate students, development and teaching of a graduate level course in his or her specialty in cell biology, and teaching general cell biology to undergraduate students. A complete curriculum vitae and a list of three references should be sent to Professor Joseph Y. Cassim, Chairman, Cell Biology Search Committee, Department of Microbiology, The Ohio State University, 484 W 12th Avenue, Columbus, Ohio 43210. Applications will be accepted until January 15, 1980.

An Equal Opportunity/Affirmative Action Employer. Qualified women, minority and handicapped persons, as well as others are encouraged to apply. W514(A)

Laser Scientist

The Rutherford Laboratory is one of the Science Research Council's principal establishments for advanced work in physics and is also one of Europe's leading centres for laser research.

The Laser Division has a vacancy for a Research Associate to work in an existing program of gas laser development. The present emphasis in this work is on the development of efficient, high power rare-gas halide lasers excited by fast electric discharges of high voltage electron beams. These lasers are of interest for use in laser-plasma interaction studies. A Ph.D in physics or a related area is required and previous experience in lasers or gas discharge physics would be an advantage.

The post is tenable for 3 years on the salary scale £5,273-£7,086 (with a further pay rise on 1.1.80).

Benefits include a Non-contributory Superannuation Scheme and generous sick leave and annual leave allowances.

Write or telephone for an application form to:

Jane Griffiths in the Personnel Group quoting ref: VN 858. Rutherford and Appleton Laboratories, Chilton, Didcot, Oxon OX11 0QX. Tel: Abingdon (0235) 21900 ext. 510.

The closing date for applications is 30th November, 1979.

3659(A)

Petroleum Geochemist

Worldwide Exploration Activities

We need a Geochemist, to work in our Geochemical Review Group, who has several years' experience of the application of geochemical techniques to exploration problems in the oil industry.

Part of our Exploration Division, this Group is concerned with the integration of geochemical and geological data into a regional framework presently covering the U.K., Ireland, the Middle East, Malaysia and North America. Some travel to these areas may be required.

Applicants should be aged 25 to 35 years with a good Honours Degree in Geology and a PhD in Organic Geochemistry.

Vacancies also exist within our Geochemical Branch for applicants similarly qualified but with less experience.

Starting salaries will be dependent on age and experience, with excellent benefits including non-contributory pension scheme, four weeks' holiday and relocation assistance, where appropriate.

Please write with full details of qualifications and experience to:

Mrs. L. Fuller, BP Research Centre, Chertsey Road, Sunbury-on-Thames, Middlesex TW16 7LN.

BP Research Centre Sunbury on Thames BP

3667(A)

The University of New England Armidale — Australia

Professor of Botany

The University invites applications for the Chair in Botany. The appointee will be Head of the Department for five years in the first instance.

The Department offers courses for internal students in the Faculties of Science, Resource Management, and Rural Science, and for internal and external students in the Faculty of Arts.

Full particulars can be obtained from the Staff Officer, University of New England, Armidale, New South Wales, 2351, Australia, with whom applications, which should include the names and addresses of three referees, close on 31st December, 1979, stating position No. 442.

Informal enquiries may be directed in the first instance to the Dean, Faculty of Science. 3657(A)

UNIVERSITY OF THE WITWATERSRAND
Johannesburg
South Africa

DEPARTMENT OF APPLIED MATHEMATICS

This Department consists of three divisions: Applied Mathematics, Computer Science and Statistics. It has active groups in various areas of Applied Mathematics, Computer Science and Operations Research.

Applications are invited from suitably qualified persons for appointment to the following posts:

(1) *Chair of Statistics*
The successful applicant will be Head of the Division of Statistics and will be expected to lead the Division in its teaching and research efforts. The applicant's own field of interest may be in Mathematical Statistics, Applied Statistics or Operations Research.

(2) *Chair of Computer Science*
The successful applicant will be Head of the Division of Computer Science and will be expected to lead the Division in its teaching and research efforts.

(3) *Chair in the Field of Mathematics*
This Chair falls within the Department of Applied Mathematics and applications will be considered from academically well qualified candidates who have active research interests in any of the Mathematical Sciences.

Salary attached to these posts will be within the range R15 000 — R18 000 per annum. A limited amount of private work will be allowed. (£1 = R1.77 approx).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged by 30 November 1979. Applicants should indicate the post for which they are applying. 3705(A)

PLYMOUTH POLYTECHNIC

School of Enviromental Sciences
RESEARCH ASSISTANT MICROBIOLOGY

To work on the isolation and characterisation of Toxins from fish pathogenic bacteria.

Applicants should hold a degree in Microbiology Biochemistry or related subject and will be expected to register for a higher degree. The post would also be suitable for a post-doctoral candidate.

Research assistant are normally required to register for a higher degree, although post doctoral applicants will be considered. Appointments are for a period of two years with a possibility of a third year (fixed term contracts).

Salary will be £3480 for postgraduates or £3780 post-doctoral (or equivalent) with annual increments of £150.

Application forms returnable by 30th November 1979 and further particulars can be obtained from the Personnel Officer, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA.

3719(A)

NATIONAL UNIVERSITY OF LESOTHO

Applications are invited for the

CHAIR OF BIOLOGY

tenable from January 1980. Candidates should have a higher degree and at least 10 years university teaching, research and administrative experience. Candidates working in any area of biology, applicable to a montane, grassland environment may apply but it is unlikely that an appointment will be made in the fields of genetics, entomology, biochemistry or plant physiology which are already covered by present members of staff. Preference will be given to candidates whose teaching and research experience is in ecology. Those who responded to the previous advertisement need not reapply, their applications will be considered alongside new ones. The appointment will be on permanent terms for local applicants or on contract terms for limited periods of two to four years for expatriate applicants in the first instance.

Salary: R8580 — R8790 — R9000 pa (£1 sterling = R1.78). The British Government may supplement salary by £3870 pa (sterling) for married appointee or £2766 pa (sterling) for single appointee (presently under review and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; baggage allowance; superannuation is non-contributory for appointees on permanent Terms of Service, while appointees on short contract terms receive 25% gratuity in lieu of superannuation for the first two years of the contract rising to 27½% and 30% for each subsequent and similar period of service; 15% inducement allowance for those not qualifying for any supplementation scheme, unless married to another supplemented member of staff; subsidised accommodation is available; education allowance for expatriates; overseas leave every 2 years; study leave every four years.

Detailed applications (2 copies) with curriculum vitae and naming three referees should be sent by 10th December, 1979 to the Assistant Registrar (Appointments), NUL, Roma, Lesotho, Africa. Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P ODT. Further particulars may be obtained from either address. 3685(A)

IRAM
Institut de Radio Astronomie Millimétrique

requires a

PHYSICIST/RADIO ASTRONOMER

with broad background and experience in the use and/or development of radio interferometers, to participate in several of the following activities:

The design and development of surface measurements of high precision antennas by holographic and/or interferometry methods.

The design and development of focal systems for multifrequency observations, polarization measurements, with consideration of problems of standing waves and "Baseline ripple".

The analysis of the possible use of various physical devices such as fast logical circuits, quasi-optical components, acousto-optical systems, fibre optics.

The development of observing and data reduction methods for radio astronomical interferometers with little known or unknown phase.

The station of work will be GRENOBLE.

Minimum base salary is about: 7500 F.F./month.

Applicants should send curriculum vitae with a list of publications and the names of a few references to: Institut de Radio Astronomie Millimétrique, 53 Avenue des Martyrs, 38026 Grenoble CEDEX, France. W507(A)

CSIRO AUSTRALIA

Chief of Division Division of Manufacturing Technology

Applications are invited from scientists and engineers, who have an established record of personal research achievement and leadership, for the position of Foundation Chief of the newly formed Division of Manufacturing Technology. The new Division has been established by separating from the Division of Materials Science its staff and resources engaged in the research detailed below.

CSIRO is Australia's largest and most comprehensive research organisation having approximately 7,000 employees of whom 2,400 are research and professional scientists. Its broad charter covers research into problems of primary and secondary industries and such fields of community interest as human nutrition, the environment and the development and use of natural resources. The organisation's 43 scientific research divisions and units, which are dispersed throughout Australia, have recently been grouped into 5 institutes. Each institute is headed by a Director. The Division of Manufacturing Technology is one of the divisions within the Institute of Industrial Technology. Other component divisions of the Institute are the Divisions of Applied Organic Chemistry, Building Research, Chemical Technology, Mechanical Engineering, Protein Chemistry, Textile Industry and Textile Physics.

The Division of Manufacturing Technology has laboratories in Melbourne and Adelaide and the future establishment of laboratories in Sydney is envisaged. The divisional headquarters will be located in either Melbourne or Adelaide. The Executive will determine the location in consultation with the Chief-Designate. The current staff of the Division is 67 of whom 34 are professional scientists. The objectives of the Division are to develop new and improved fabrication methods, to maximise the economic use of materials and to improve their properties having regard to the technology of their use. There is particular emphasis on the improved processing and utilisation of indigenous raw materials.

The present programs aim at providing the technological base for continuing economic viability in areas of metal fabrication which are fundamental to a wide range of industrial activities. The programs are carried out in conjunction with industry groups to ensure maximum external input and ease of implementation. The inter-disciplinary approach emphasises technological advancement through the development of the basic principles of physics, metallurgy and engineering. The present programs encompass a range of high volume, high precision forming processes from both liquid and solid metal, the wear and corrosion of components and their prevention by a variety of surface treatments. It is intended that the divisional programs will be expanded to include a wider range of materials and manufacturing activities.

In appointing a Foundation Chief, the CSIRO Executive wishes to establish the Division of Manufacturing Technology as a prime centre of expertise for the Australian manufacturing industry. This will require the Chief to have proven ability in identifying long-term industrial problems, and in technology transfer and implementation as well as research leadership.

A Chief of Division is responsible for the development, scientific direction and integration of the research programs of the Division and for the development of active collaboration with industry bodies and research associations. The Chief of the Division of Manufacturing Technology will be expected to have a close working knowledge of the manufacturing industry in Australia and its research and development requirements.

The salary for the position is negotiable but will not be less than Dols Aust 35,474 p.a.

The terms of the appointment will be discussed with the short-listed applicants at the inverview. The appointee would be eligible to contribute to the Commonwealth Superannuation Scheme.

Further information on the Division is in a booklet available on request from the Secretary, CSIRO, at the address below.

Dr H.W. Worner, Director of the Institute of Industrial Technology and Chairman of the Appointment Committee, who may be contacted at the address below, will be available for discussions concerning the appointment with applicants and other interested parties.

Applications stating full personal and professional details and names of at least two professional referees and quoting reference number 1600/001 should reach The Secretary, CSIRO, PO Box 225, Dickson, A.C.T. 2602, Australia by 14 December, 1979.

3714(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Applied Physics Sydney

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees — 2400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

Field: Non-Destructive Testing

General: The Division has the role of maintaining and developing Australian standards of measurement of physical quantities. It undertakes problems of research in related areas, and is involved in the solution of specific problems relating to industry. The Division is located in a large new laboratory complex at West Lindfield. In addition there are branch laboratories in Adelaide and Melbourne.

Duties: To assume a major role in the establishment of a new research activity in the field of non-destructive testing as part of the Division's programme of assistance for Australian manufacturing industry. Emphasis will be placed on using ultrasonic techniques to extend the present sensitivity limits for the detection of defects and to improve the precision with which defects can be characterized. The project will have a close relationship with existing research activities, including studies in vibration, acoustics, solid state physics and optical holography.

Qualifications: A PhD in Physics or Engineering, or equivalent qualifications, with experience in ultrasonics. Some knowledge of appropriate areas of metallurgy would be an advantage.

Salary: Research Scientist/Senior Research Scientist: $A15,916 — $A23,122 pa.

Tenure: A fixed term appointment of three to five years may be negotiated. Commonwealth Superannuation is available.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 750/781 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 7th December 1979.

Applications in U.S.A. and Canada should be sent to: — The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3687(A)

Directorate of Scientific Services

Assistant Microbiologist

Max Salary £6,900 p.a.

To work at the New River Head Laboratories, Rosebery Avenue, EC1.

Will be required to undertake microbiological examination of water at all stages of treatment, research and development work.

Applicants should hold a degree in a natural science and have considerable experience in the microbiological analysis of water for public supply.

Application forms available from the Assistant Director (Personnel — Staff), New River Head, Rosebery Avenue, London EC1R 4TP or telephone 01-837 3300 Ext. 2024. 3672(A)

Thames Water

THE QUEEN'S UNIVERSITY OF BELFAST

LECTURESHIP IN BIOCHEMISTRY

A lectureship in the Department of Biochemistry is available from 1st January, 1980, or such other date as may be arranged. The lecturer will be expected to teach at various levels and to engage in research, preferably collaborative, in fields already active in the Department, details of which are set out in a departmental prospectus which is available. Initial placing, which will depend on age, experience and qualifications, will be made at one of the first three points on the scale for lecturers; £4335, £4623, £4911 rising to £8994 with contributory pension rights under the FSSU/USS. The appointment will be subject to a period of probation of up to three years.

Further particulars are available from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. Closing date: 23rd November 1979. (Please quote Ref: 79/N). 3671(A)

POSITIONS ARE AVAILABLE in the Department of Biochemistry for teacher-researchers in molecular genetics and in the metabolism and structure of cell surface glycoproteins or the components of the cytoskeletal system. The positions are tenure track and are at the level of assistant professor. Interested candidates should contact: Dr M Waite, Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salam, North Carolina 27103. Telephone (919) 727 4255. An Equal Employment Opportunity/Affirmative Action Employer. W515(A)

GRIFFITH UNIVERSITY
Australia

SCIENCE, TECHNOLOGY AND SOCIETY

READER/SENIOR LECTURER
(re-advertisement)

Applications are invited from qualified men and women for the senior academic appointment in the area of Science, Technology and Society (STS) within the School of Science. The appointee will have had extensive training and research experience in one or more aspects of science policy. Applications from persons with interests in Technological and Social Change and/or Science and Government in either the developed or developing worlds would be particularly welcome.

Undergraduate students in the School are required to study STS for a quarter of their time in first year and optional courses are available in later years. A coursework M.Sc. programme has taken its third cohort of students. The appointee will have considerable responsibilities in the teaching programmes and in the academic development of STS within the School.

A Science Policy Research Centre (SPRC) has been established and the appointee will assume the position of Director of the SPRC, and as a consequence will have a reduced teaching load. Funds for a part-time research assistant are provided initially.

Salary Scales: Reader — $A28,809; Senior Lecturer — $A21,858-$25,477. The University also meets the cost of reasonable removal expenses.

Further details and the method of application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. Applications close on *30 November 1979.* 3670(A)

TECHNICIAN/ RESEARCH OFFICER

to assist our Hormone Biochemistry Laboratory in the use of biochemical and cell culture techniques to investigate the action of steroid hormones in regulating the growth of breast tumour cells.

HNC/Degree. Salary range £4122 to £5949. For further information and application form write or telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 on 242-0200 ext 305, quoting reference HB1. 3674(A)

IMPERIAL COLLEGE OF SCIENCE
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL RESEARCH FELLOW

Applications invited for a post-doctoral research assistantship to work on the magnetic properties of metal porphyrins in solution. The post is for two years, starting as soon as possible. Salary on Scale £4333 — £7521 plus £740 London Allowance.

Applications enclosing a curriculum vitae and the names of two referees should be sent to Dr D.F. Evans, Department of Chemistry, Imperial College of Science, London SW7. 3718(P)

UNIVERSITY OF REGINA
DEPARTMENT OF BIOLOGY AND CHEMISTRY
POSTDOCTORAL POSITIONS

A two year postdoctoral position (salary approx. $14,500) is available in a molecular biology-biochemistry laboratory (headed by Dr H. Bertrand) studying the genetic control of the biogenesis of mitochondria respiratory complexes in *Neurospora crassa*. The laboratory is well equipped for research on the assembly and function of submitochondrial complexes, restriction mapping of DNA, protein and nucleic acid chemistry and for genetic manipulations.

A second postdoctoral position ($1,000 Cdn/month; ending June 30, 1979 with the possibility of an additional year pending budgetary approval) is available for research on the regulation of pyrimidine and arginine metabolism in *Salmonella typhimurium* (headed by Dr R.A. Kelln). Research activities will include mutant isolation, genetic mapping, nucleotide pool quantitation enzyme assays and protein and nucleic acid chemistry.

Interested individuals should be available as soon as possible and should have completed a PhD degree in biochemistry, genetics or physiology.

Applications, in the form of curriculum vitae with names and addresses of three references should be sent to:

Department of Biology (or Chemistry) University of Regina, Regina Saskatchewan, Canada S4S 0A2. W487(A)

RESEARCH SCIENTIST
Corrosion Science
Electrochemistry
Metallurgy
Physics

This senior position in the FONTANA CORROSION CENTER will be of interest to persons who hold a PhD degree in metallurgy, physics, physical chemistry, or corrosion science, and who have an established record of scientific research. The successful applicant will direct research on a wide variety of fundamental and applied problems.

Interested persons should send résumés and the names of three referees to Prof. D D Macdonald, Director.

Fontana Corrosion Center, Dept. of Metallurgical Engineering, The Ohio State University, 116 West 19th Avenue, Columbus, Ohio 43210, USA.

An equal opportunity/affirmative action employer. W513(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF CHEMISTRY

CHAIRS OF ORGANIC CHEMISTRY

Applications are invited for appointment as Professor of Chemistry with special reference to Organic Chemistry, following the retirement on 31 December 1980 of Professor A J Birch, FAA, FRS. A second chair has recently been established and may be filled at the same time.

Founded in 1967, the Research School of Chemistry is an integrated School without departments. A professor who is not Head of School can devote almost his whole effort to research, being expected only to attend to minor administration in the subject area as a whole and to play some part in University affairs. However, a professor is expected, if invited by the Council, to serve a three-year term as Head of School at some stage. The Dean as Head of School is chosen by the Council from amongst the professors in the School. The Dean acts with the advice of the Faculty and the Faculty Board, but has the executive power to act on his own behalf. The present Dean is Professor D P Craig, FAA, FRS.

The School has a total academic staff of seventy-four, of whom fourteen hold tenured appointments. There are four professors (Professors A J Birch, D P Craig, B G Hyde and A M Sargeson) and three professorial fellows (Dr M A Bennett, Dr J Ferguson and Mr R W Rickards). The School has no responsibility for undergraduate teaching. It carries out research and teaches graduate students, is well equipped by modern standards, and is well supported by technical and administrative staff.

In the Organic area there are five tenured staff members leading independent research groups (Professor A J Birch, Mr R W Rickards, Dr J K MacLeod, Dr L N Mander and Dr L Radom); there are at present twenty-two research fellows and eight PhD students.

The normal salary level is $A36,253 per annum. Current exchange rates $A1:50p: $US1.13. Superannuation benefits are available. The University meets reasonable travel and removal expenses for the appointee and family and also assists in providing suitable housing.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Further information about the position may be obtained from the Registrar, P.O. Box 4, Canberra, ACT 2600, Australia, or from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on *11 January 1980.* 3660(A)

U M I S T

POST-DOCTORAL RESEARCH IN CORROSION

Aplications are invited from suitably qualified scientists to undertake research in a number of areas, including:

(a) The effect of ion implantation on the corrosion, oxidation, wear and other surface properties of copper.
(b) The mechanisms of the inhibition of the corrosion of aluminium.
(c) The etching of aluminium.

Prior experience in the field of corrosion is desirable, but not essential. Salary will be on the scale £4333 — £7521 per annum.

Enquiries for further information should be addressed to Professor G C Wood, Corrosion and Protection Centre, UMIST.

Requests for application forms, quoting reference CPC/229, should be addressed to the Registrar, UMIST, P.O. Box 88, Manchester M60 1QD. The closing date for this post is 22 November 1979. 3664(A)

RE-ADVERTISEMENT
MEDICAL RESEARCH COUNCIL

VIROLOGY UNIT

ENZYMOLOGIST
PROTEIN BIOCHEMIST
and
NUCLEIC ACID BIOCHEMIST

Two vacancies exist for post-doctoral research scientists with experience in enzymology/protein biochemistry or nucleic acid biochemistry to work on a variety of molecular biological problems involving Herpes Simplex Virus and other mammalian virus systems.

Previous experience with virus systems is advantageous but not esential.

Each appointment will be for three years. Salary on an appropriate point on Grade 2 of the non-clinical scientific staff scale (£4,333 to £6,932). Applicants should send a Curriculum vitae giving full details of qualifications and experience and the names and addresses of three professional referees to: Professor J.H. Subak-Sharpe, Institute of Virology, University of Glasgow, Church Street, Glasgow, G11 5JR by 15th December, 1979. 3712(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY
Babraham, Cambridge
CB2 4AT

A vacancy, financed by the M.R.C. for 3 years, exists for a Biochemist to conduct research into the structure of Major Transplantation Antigens in relation to their biological properties. Salary in Scientific Officer (£3,289 — £5,085 at present. £3,591 — £5,486 from 1.1.80 p.a.) or Higher Scientific Officer (£4,727 — £6,211 at present. £5,097 — £6,737 from 1.1.80 p.a.) scale according to experience.

Qualifications for S.O.: Degree in an appropriate subject or H.N.C. with relevant experience; at least five years relevant post qualifying experience required for H.S.O. (preference for Ph.D.). Non-contributory superannuation scheme.

Applications quoting reference J.C.H.4. to the Secretary of the Institute by 23rd November 1979. 3707(A)

SANDOZ

Pathology

Sandoz Limited is a major Swiss-based multinational pharmaceutical company with a large, modern and well equipped Toxicology Institute in Basle, Switzerland. We are looking for suitable candidates to fill 2 posts that have recently become vacant in our Pathology Department.

A. Head of Section

Job Description: Overall responsibility for the activities of the Pathology Section which comprises a staff of about 20 members including 4 pathologists (Medical Doctors and Veterinarians). The routine activities of the section include the planning, supervision, evaluation and reporting of the post mortem aspects of chronic toxicity studies conducted on animals, including carcinogenicity tests. Since academic members of the Department are also encouraged to pursue their own research interest and to investigate the mechanisms by which drugs induce their toxicity, the Section Head is also expected to be in a position to contribute and encourage such activities. There will be ample opportunities to attend scientific conferences. The Head of Pathology is directly responsible to the Head of Toxicology.

The Ideal Candidate: Will be 30-45 years of age, possess a medical or veterinary qualification (preferably with a higher degree in Pathology) and will have considerable practical experience of laboratory animal pathology. Administrative ability is required for this position as is excellent written and spoken English. Previous industrial experience and a knowledge of German would be advantageous.

B. Pathologist

Job Description: The planning, supervision, evaluation and reporting of the post mortem aspects of chronic toxicity studies conducted on animals (especially carcinogenicity tests on rats and mice). In addition, the investigation of the mechanism by which drugs induce their toxicity. There will be ample opportunities to pursue individual scientific interests, to attend scientific conferences and for the periodic updating of expertise through stays in external laboratories (universities, etc). The pathologist will have 2 histology laboratories and attendant staff (5 technicians) at his disposal. He will be directly responsible to the Head of Pathology.

The Ideal Candidate: Will be 30-45 years of age and have a degree in Medicine, Veterinary Medicine or an appropriate biological discipline. In addition, he will have experience of laboratory animal pathology, preferably with specialized knowledge of tumor pathology and its diagnosis. Organizational talent is required for this post and previous industrial experience and some knowledge of German would be advantageous.

General Information:

- Removal expenses to Switzerland will be defrayed by Sandoz Ltd.
- The firm runs a contributory pension scheme.
- The Toxicology Institute is located in pleasant rural surroundings some 6 miles from the centre of Basle.

Interviews of suitable candidates for both posts will be held in London in mid-November. Short list candidates will be subsequently invited to Basle.

Applications, which should be made in writing, should be accompanied by a comprehensive curriculum vitae and the names of two referees.

Apply to: The Personnel Department, Ref. 1244, Sandoz Ltd., CH-4002 Basle, Switzerland.

W458(A)

Boehringer Mannheim GmbH is a pharmaceutical company strongly engagend also in the field of biochemical research reagents.
Applications are invited for the position of

BIOCHEMIST

in our R&D laboratories in Tutzing/South of Munic. This position in the Molecular Biology department offers a opportunity to join a young and enthusiastic team involved in the challenging developmental research towards the production of enzymes acting on nucleic acids.

The successful candidate will already have practical skills and experience in analytics, application and/or isolation of these enzymes. Knowledge of the German language is required.

The firm offers excellent conditions of employment.

For further information please send your application with scientific curriculum vitae to

BOEHRINGER MANNHEIM GMBH
Biochemica Werk Tutzing
— Personalabteilung —
Bahnhofstraße 9-15, D-8132 Tutzing

mannheim boehringer
diagnostica therapeutica

W512(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF GEOLOGY

Prior to application, further information (including application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, ext. 431.

The closing date for receipt of completed applications is Thursday, 6th December, 1979. 3623(A)

IMPERIAL COLLEGE (University of London)

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

to work with Dr Bruton on the structure and interactions of aminoacyl-TRNA synthesis. Experience in protein or nucleic acid chemistry is highly desirable. Salary in the range £5,073 to £8,261 (inc). The post is funded by the SRC and is for 2 and three quarter years.

Applications, including a full c.v. and the names and addresses of two academic referees should be sent to Dr Chris Bruton, Department of Biochemistry, Imperial College, London SW7 2AZ. 3683(A)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited from neurobiologists with previous research experience in Neurophysiology/Pharmacology for a post to study the effects of long-term antidepressant drug administration on monoamine release and receptor activity in rat brain using *in vitro* techniques. The appointment is for three years commencing as soon as possible. Initial salary £4,333.

Applicants should send their curriculum vitae and names of two referees to Dr C. A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH from whom further information may be obtained (0602) 700111 Ext. 3297. 3679(A)

UNIVERSITY OF LONDON

INSTITUTE OF NEUROLOGY

Applications are invited for a post-doctoral position in a Research Group supported by an MRC programme grant working on neurochemical and behavioural effects of environment, nutrition and experimental disease (liver, kidney failure, etc). Salary between £5,073 — £8,261 (incl. London Weighting).

Curriculum vitae with names of 2 referees by 7 December to Professor G. Curzon, Institute of Neurology Labs, 33 John's Mews, London, WC1. 3706(A)

UNIVERSITY OF MANCHESTER

RESEARCH ASSOCIATE in MEDICAL BIOCHEMISTRY

Applications are invited from post-doctoral candidates for this post (available for three years) to work on glycoproteins and proteoglycans in collagenous structures of blood vessels. Some experience in the fields of connective tissue glycoconjugates, immunolocalisation or cell culture would be an advantage. Starting salary in the range £4,333 — £4,910 p.a.

Applications with full curriculum vitae and the names of two referees should be sent, as soon as possible, to Professor D S Jackson, Department of Medical Biochemistry, University of Manchester Medical School, Manchester M13 9PT. 3709(A)

ROYAL HOSPITAL FOR SICK CHILDREN

DEPARTMENT OF PATHOLOGY

RESEARCH ASSISTANT

Graduate Biochemist required for work on the expression of enzyme markers in malignant disease. Opportunity for undertaking higher degree. Salary according to nationally agreed scales.

Applications giving the names of two referees should be sent as soon as possible to The Secretary, Department of Pathology, Royal Hospital for Sick Children, Edinburgh, EH9 1LF. Tel: 031 667 1991 ext 270. 3713(A)

KING'S COLLEGE LONDON

TECHNICIAN GRADE 4

To be in charge of the day-to-day running of practical classes for medical, dental and B.Sc. students in the Anatomy Department. There are new Faculty Laboratories serviced by a Departmental Preparation Room. O.N.C. or equivalent qualification required with a minimum of 7 years experience including histology.

The successful applicant will be responsible for the expansion of histology teaching material. Salary on scale £4212 p.a. rising to £4730 p.a. inclusive (under review). 5 weeks annual holiday. Superannuation scheme. Interest-free loans for annual rail reason tickets. Apply in writing with full details to: The Head Clerk (Ref: 221734N), King's College London, Strand, WC2R 2LS. 3703(A)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

Chemistry and biochemistry Division (Physical Methods Group)

PHYSICIST/PHYSICAL CHEMIST (Scientific Officer)

A Physicist/Physical Chemist is required to join a newly-constituted team to study the physicochemical properties of dispersions and gels in foods.

Qualifications: First or Upper Second Class Honours degree in Physics, Chemistry or biochemistry. Previous experience in surface chemistry or in the use of physical methods in Biology will be an advantage.

Salary: From 1st August 1979: on a scale £3,289 — £5,085. From 1st January 1980: on a scale £3,591 — £5,486.

Non-contributory superannuation scheme at 18 yers and over. Five day working week and flexible working hours scheme operated. Transport facility available mornings and evenings.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting ref: 79/10.

Closing date 26th November 1979. 3717(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

DEPARTMENT OF BIOLOGY

POST-DOCTORAL POSITION

available immediately to study Host-pathogen interaction in plants. Training in Biochemistry and Plant Pathology preferable; $11,800 per annum or more depending on experience.

Applications, including curriculum vitae and names of three referees, should be addressed to Dr Arya K. Bal, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9; or call (709) 753-1200, Ext. 2694. 3686(A)

FACULTY POSITION

Organic Chemistry

Applications are invited for a tenured senior appointment which the Department of Chemistry at Stanford University expects to be able to make in the near future. Applicants must have outstanding research credentials in organic chemistry and a strong commitment to teaching undergraduates as well as graduates.

In applying, send curriculum vitae and bibliography to: Professor K.O. Hodgson, Department of Chemistry, Stanford University, Stanford, California 94305. Stanford is committed to Equal Opportunity through Affirmative Action in employment and we are especially eager to identify women and minority persons with appropriate qualifications.

Applications should be received by 15 December, 1979. W516(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL

DEPARTMENT OF HISTOCHEMISTRY

RESEARCH/SCIENTIFIC OFFICER

required to work on problems connected with the localisation and dynamics of peptide hormones and neuroendocrine regulation in man and experimental animals.

Applicants should be graduates (or equivalent) and must have experience in some or all of the following techniques: histology, histochemistry, electron microscopy, antibody production immunostaining.

The suitable candidate will have opportunity for specialising in a particular area and/or will be able to apply for a higher degree.

Salary, dependent on qualifications and experience, initially between £4,000 and £6,000 a year.

Further particulars and application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W1 0HS quoting reference number 6/222/N. 3682(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL
INSTITUTE OF TERRESTRIAL ECOLOGY

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

A Scientific Officer/Higher Scientific Officer is required by the Institute at Craighall Road, Edinburgh, to investigate the population dynamics and spatial distribution of zooplankton in shallow lakes.

QUALIFICATIONS

Candidates should have at least a 2.1 honours degree in Zoology, Ecology, Limnology, Mathematical Biology or a similar subject. Relevant experience is desirable together with an interest in freshwater biology. Candidates for HSO should have at least 2 years post graduate experience in a relevant subject for those with an Honours Degree or 5 years experience with HNC/HND. Mathematical ability and experience are essential.

SALARY AND CONDITIONS OF SERVICE

Scientific Officer:
£3,289 – £5,085 pa. From 1.1.80: £3,591 – £5,486 pa.
20 days paid annual leave

Higher Scientific Officer:
£4,727 – £6,211 pa. From 1.1.80: £5,097 – £6,737 pa.
22 days paid annual leave

Up to 22 days annual leave plus 10½ days public and privilege holidays.

Starting pay according to age, qualifications and experience.

There is a non-contributory superannuation scheme.

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from:
Establishments Section, Institute of Terrestrial Ecology, 68 Hills Road, Cambridge, CB2 1LA. Telephone (0223) 69745.

Closing date for completed applications is: 23 November 1979. Reference: 64.
3662(A)

UNIVERSITY OF LONDON
KING'S COLLEGE

DEPARTMENT OF CIVIL ENGINEERING

A RESEARCH ASSISTANT

is required to join a collaborative project with the Transport and Road Research Laboratory on the analysis and prediction of the structural behaviour of road pavements under repeated loading.

The appointment is likely to be at Post-Doctoral level and is for two years in the first instance. Adequate background and experience in analytical and numerical techniques is essential. Depending on age and experience, the starting salary for a post-doctoral worker will be at one of the first three points on the 1A scale (ie £4333, £4622 or £4910 pa) plus a London Allowance of £740 pa.

Applicants having good relevant experience, but without a doctorate, may also be considered. Applications, giving a brief curriculum vitae and the names and addresses of two referees should be sent to Professor J W Dougill or Professor K Nash, Department of Civil Engineering, King's College, Strand, London WC2R 2LS, either of whom will be pleased to give further details of the appointment.
3702(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF PLANT BIOLOGY

Applications are invited for the post of

DEMONSTRATOR

in the Department of Plant Biology. The appointment will be for a period of three years from 1st January, 1980. Candidates should have appropriate postgraduate experience and preference may be given to ecologists who have shown special interest in population biology. Duties will include teaching undergraduates and helping with field classes.

Salary will be at an appropriate point on the Grade 1B (Bar) scale: £3,775 – £5,488 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

No forms of application are issued, but applications (3 complete copies), giving full details of age, qualifications, experience and present salary, together with the names and addresses of 3 referees, should be lodged with the Senior Assistant Registrar, The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, not later than 30th November, 1979. (Preliminary enquiries may be made directly to the Head of Department of Plant Biology.) Please quote reference N. 3668(A)

GEORGE WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORATE ASSOCIATE

To work in the areas of prostaglandin metabolism or cultured cell-lipoprotein interactions. Salary will be $14,000 per annum.

Applications consisting of a curriculum vitae and names of two referees should be sent to Director, Postgraduate Appointments, Biochemistry Department, George Washington University School of Medicine, 2300 Eye Street, NW, Washington DC 20037, USA. (An Equal Opportunity Employer)
W521(A)

AGING AND MOLECULAR BIOLOGY

POSTDOCTORAL POSITIONS AVAILABLE

to study

i) aging of the transcription and translation processes in vivo or ii) bacteriophage development in anucleate cells.

Candidates should have experience in either enzymology, preferably RNA polymerase, in vitro transcription/translation systems, characterisation of DNA replication intermediates or bacterial/phage genetics. Applications with full C.V., research experience and names of referees to Dr J N Reeve, Dept. Microbiology, Ohio State University, Columbus, Ohio 43210.

An Equal Opportunity/Affirmative Action Employer. Qualified women, minority and handicapped persons, as well as others, are encouraged to apply.

CSIRO AUSTRALIA

Assistant Chief

Division of Human Nutrition Adelaide

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees – 2400 of who are research and professional scientists – located in Divisions and Sections throughout Australia.

General: Research in the Division of Human Nutrition is concerned with studies of nutritional processes with a view to identifying the existence and health consequences of nutritive imbalances and deficiencies in Australian diets. Its research includes experimental studies in inorganic nutrition and in metabolism and digestion, and epidemiological and behavioural studies with emphasis on the relations between nutrition, life-style, and human health.

Duties: To assist the Chief of the Division, Dr B S Hetzel, in the planning and development of research priorities. The appointment is sought of a senior scientist of proven achievement with a strong background in a laboratory discipline related to nutrition, preferably in some aspect of lipid biochemistry where there is already an active research group in the Division. Previous administrative experience is essential. The opportunity and resources will be provided to enable the appointee to pursue an active research role.

Tenure: This is an indefinite appointment which carries superannuation benefits, subject to normal conditions. However, the role of Assistant chief is offered for a term of three years in the first instance. The situation will then be reviewed to determine whether the appointee should continue as Assistant Chief for a further period, or as a senior research worker.

Salary: Principal Research Scientist/Senior Principal Research Scientist: $A23,991 – $A31,507 pa. Appointment at a higher level will be considered for a quite outstanding applicant.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 250/280 should reach:–

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 7th December 1979.

Applications in U.S.A. and Canada should be sent to: – The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3690(A)

EDITORIAL ASSISTANT

Applications are invited from graduates in biochemistry or a related subject for the post of Editorial Assistant in the Editorial Office of the Biochemical Society in London.

Duties consist mainly in preparing typescripts for the printer and proof-reading for the various scientific publications of the Society. Applicants should have an interest in the presentation of the scientific text, although editorial office experience is not essential as training will be given.

The commencing salary will be £5,000 pa. Please apply in your own handwriting, with the names of two referees, to the Editorial Manager, The Biochemical Society, 7 Warwick Court, London WC1R 5DP.
3676(A)

ANGLO-EUROPEAN COLLEGE OF CHIROPRACTIC LTD

LECTURER

Applications are invited for the post of lecturer in Pathology, Microbiology and Haematology, as from 1st January, 1980.

The appointed person will be required to be responsible for the running of a small Clinical Haematology Laboratory in the College Clinic.

Applications together with curriculum vitae and the names of three referees to be sent to the Dean, Anglo-European College of Chiropractic, 1 Cavendish Road, Bournemouth, Dorset, BH1 1QX, as soon as possible. 3642(A)

CSIRO AUSTRALIA

Research Scientist

Division of Food Research Food Research Laboratory North Ryde

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees — 2400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

Field: Electron Microscopy.

General: The food Research Laboratory at North Ryde, NSW is part of the Division of Food Research. The research programmes of the Division include many aspects of the chemical, physical and biological sciences related to the handling, processing and storage of foods and to the needs of the food industry and the consumer. The Food Research Laboratory, which has a total staff of 175, is working on sensory aspects of foods, food structure, safety and quality, plant physiology and food technology.

Duties: To carry out investigations of biological and physical materials using light and electron microscopical techniques. The appointee will be encouraged to collaborate with other scientists on a diverse range of problems at the Laboratory and will be expected to develop an independent research programme.

Qualifications: A PhD degree or equivalent with experience of light microscopic, transmission and scanning electron microscopic and freeze-fracture techniques.

Salary: Research Scientist/Senior Research Scientist: $A15,916 — $A23,122 pa.

Tenure: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 300/745 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 7th December 1979.

Applications in U.S.A. and Canada should be sent to: — The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3689(A)

A POSTDOCTORAL RESEARCH FELLOW

is required in early 1980 to work for 1-2 years on a project sponsored by the Deutsche Forschungs-gemeinschaft on Cellular Interactions and early membrane events in mitogenic lymphocyte stimulation.

The salary is in the range of 40,000 Deutsche Mark/year.

Applications, consisting of full curriculum vitae, list of publications, and naming 2 referees should be addressed to: Dr W G Bessle, Lehrstuhl Mikrobiologie II Arbeitsgruppe Immunologie, Auf der Morgenstelle 28, D 74 Tuebingen, Fed. Rep. of Germany. W508(A)

RESEARCH ASSOCIATE (M.D. or Ph.D.)

To participate in a biomedical research project at Washington University School of Medicine involving regulation of protein synthesis. Experience in nucleic acid biochemistry and *in vitro* protein synthesis required.

Annual salary $12-15,000 plus benefits. Excellent working conditions. Apply to Carol Cassidy, Job Service Missouri Division of Employment Security, 4645 Gravois Ave., St Louis, Mo. 63116. Equal Opportunity Affirmative Action Employer M/F/H. W511(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory appointment:

PROFESSORSHIP OF HORTICULTURE

Prior to application, further information (including application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, extension 431.

The latest date for receipt of completed applications is Thursday, 13th December 1979.

3665(A)

UNIVERSITY OF RHODESIA

SENIOR LECTURESHIPS/ LECTURESHIPS

CROP SCIENCE (An Agronomist):
A vacancy exists for a suitably qualified graduate to teach courses in Crop Production to students enrolled for the degree of BSc Agriculture with Honours. Preference will be given to applicants with experience in tropical agriculture with specific reference to conditions pertaining in Zimbabwe-Rhodesia.

CROP SCIENCE:
A vacancy exists for a suitably qualified graduate to teach and conduct research in Crop Production, Plant Breeding, Plant Physiology, Plant Protection or Horticulture. Postgraduate qualifications and experience required. Knowledge of a local African language preferred. The candidate would be expected to initiate research relevant to increasing plant food productivity in the subsistence agricultural sector. Appointment initially for 3 years.

LAND MANAGEMENT (Soil Science):
Two lectureship vacancies, one in *Soil Chemistry* and one in *Soil Physics* exist in the Department of Land Management. The candidates would be expected to teach courses in their particular fields to Honours standards and to assist with other courses in soil science.

Salary Scales and Conditions of Service for Academic Posts:
Senior Lecturer
£6934 × 269—7741 × 286—9171 p.a.
Lecturer Grade I
£6289 × 237—7237 p.a.
Lecturer Grade II
£3883 × 204—4903 × 237—6088 p.a.
Approximate figures converted from Zimbabwe-Rhodesian $ to £ sterling as at 16.10.1979.
Appointment on the above scales is in accordance with qualifications and experience.

Permanent Pensionable Terms:
Family passages and allowance towards transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished accommodation is guaranteed for a period of at least three years for persons recruited from outside Zimbabwe-Rhodesia. Sabbatical and Triennial visits with travel allowances. Superannuation and medical aid schemes.

Short-Term Contracts:
Family passages and allowance towards transport of effects. Assistance with accommodation for persons recruited from outside Zimbabwe-Rhodesia.

APPLICATIONS (6 copies) giving full personal particulars (including full name, place and date of birth etc), qualifications, publications, employment and experience, present salary, date of availability and the names and addresses of three referees should be submitted to the Appointments Officer (Staff), University of Rhodesia, P.O. Box MP 167, MOUNT PLEASANT, Salisbury, Zimbabwe-Rhodesia.
Overseas applicants should send an additional copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.
Closing date for the receipt of applications is *15 December 1979.*
British subjects considering applying for posts in Zimbabwe-Rhodesia are urged to consult the Foreign and Commonwealth Office (tel: 01-233 4995) or their nearest British Consular Office. 3661(A)

nature
London
3 Dyers Buildings
London EC1N 2NR
01-831 6901
New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

FACULTY POSITION IN BIOLOGICAL SCIENCES

at the Hopkins Marine Station of Stanford University

Applications are invited for an appointment in the Department of Biological Sciences of Stanford University, to be resident at the Hopkins Marine Station in Pacific Grove, California. The position is without restriction as to rank although some preference will be given to younger candidates.

The position involves both teaching and research. The areas in which the Station wishes to expand its faculty include Cellular, Neuro- and Developmental Biology. All applications must include a curriculum vitae, a statement of research interests and a list of three people from whom letters of reference will be sought.

Applications should reach C S Pittendrigh, Hopkins Marine Station, Pacific Grove, CA 93950 by January 15, 1980. Stanford University is an equal opportunity employer. W505(A)

UNIVERSITY OF HONG KONG

LECTURESHIP IN MICROBIOLOGY

Applications are invited for a post of Lecturer in the Department of Microbiology. Applicants who are not medically qualified will also be considered.

Annual salaries (superannuable) are:
Clinical Lecturer
HK$68,580 — 72,060 — 83,340 — 87,240 — 91,320 BAR 109,920 — 114,840 — 119,760 — 124,680 — 129,600.
Non-clinical Lecturer
HK$67,920 × 4,560 — 77,040 BAR 81,600 × 4,560 — 113,520.
(£1 = HK$10.70 approx.). Starting salary will depend on qualifications and experience.

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or the Recruitment Section, Secretary's Office, University of Hong Kong, Hong Kong.

The closing date for applications is *30 November 1979.* 3663(A)

UNIVERSITY OF GLASGOW

LECTURER (NON-CLINICAL) In the University Department of Medical Cardiology Royal Infirmary

Applications are invited for the above appointment. This post would be in biochemistry with particular reference to myocardial metabolism. Duties include participation in teaching and research. The department has a close liaison with the University Departments of Biochemistry and Pathological Biochemistry in terms of research projects.

Salary will be within the range £4,333 to £6,355 of the Lecturers' scale £4,333 to £8,992, with placement according to qualifications and experience.

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (8 copies), giving the names and addresses of three referees, should be lodged on or before 7th December, 1979.

Enquiries may be made to Professor T D V Lawrie, University Department of Medical Cardiology, Royal Infirmary, Glasgow G4 0SF. In reply please quote Ref No 4537M. 3694(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF CHEMICAL PATHOLOGY

Applications are invited from graduates for a post of

RESEARCH ASSISTANT

for work on a project, funded by the S.R.C., investigating the genetic control of sexual differentiation in the mouse. Applicants should preferably have a good background in genetics, endocrinology or immunology. The appointment will be made for a fixed period of up to three years.

Salary at an appropriate point on the IB scale for Research and Analogous Staff (£3,775 to £5,488) (under review).

Informal enquiries will be welcomed by Dr A D Stewart, Department of Chemical Pathology, Old Medical School, Thoresby Place, Leeds LS2 9NL; telephone (0532) 32799 ext. 491.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 84/10/D. Closing date for applications is 29 November, 1979. 3691(A)

TRAINEE VACUUM TECHNOLOGIST

'A' LEVELS OR A SCIENTIFIC DEGREE?

Then the Daresbury Laboratory may have a vacancy to interest you.

The Laboratory is an establishment of the Science Research Council situated in the north Cheshire countryside. At present we are constructing a large tandem Van de Graaff accelerator for experiments in nuclear structure physics.

THE JOB

We need a man or woman with a scientific background to assist with R & D work assessing the suitability of commercial vacuum equipment and materials for use in vacuum systems. You would also be involved in commissioning and operating vacuum systems for the Van de Graaff; developing special vacuum control systems; and helping with high voltage breakdown studies.

QUALIFICATIONS AND EXPERIENCE

The appointment will be made at one of two levels: (a) as a Scientific Officer for which a good honours degree in physics or applied physics is required, HNC/D holders with relevant experience would be considered, or (b) as an Assistant Scientific Officer for which 4 'O' levels including English and Maths or Physics, are required. Candidates with scientific 'A' levels would be preferred.

Training will be given but some knowledge of vacuum techniques and of transistor logic systems would be an advantage.

There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

Closing date: 23rd November 1979.

Please write or telephone Warrington (0925) 65000 ext. 467, for an application form quoting reference No. DL/710/CB to:

The Personnel Officer

DARESBURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

3710(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for 2 posts of

SENIOR LECTURER/ LECTURER

in the

DEPARTMENT OF AGRICULTURE

Applicants should have a good first degree in Agriculture and experience, preferably at PhD level. Preference will be given to applicants with University teaching and research experience. Tropical agricultural experience would be an advantage. Applicants should have expertise in one or more of the following fields: agronomy of perennial crops, pasture agronomy, crop physiology under field conditions, agricultural ecology and/or crop botany.

The appointees will be responsible for courses within their fields of competence up to Honours degree level and may be required to assist in other courses and will be expected to supervise postgraduate students and carry out independent research. They may also be asked to assist with projects on the departmental farm and to undertake other relevant duties as may occasionally be assigned. Currently the department is split between Port Moresby and Lae, but from February 1981 will be unified in Port Moresby.

The appointees may be asked to teach one or two semesters in Lae initially, but will eventually be based in Port Moresby. Salary scales: Senior Lecturer K15,575 pa, Lecturer K13,725 pa (£1 sterling = K1.55). Family passages; baggage allowance; 3 year contract; a gratuity of 24% of which 5% is taxable; research support; rent-free accommodation; overseas leave; education allowances; medical aid scheme.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Assistant Secretary, Box 4820, University PO, Papua New Guinea by 14 December 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91, Tottenham Court Road, London, W1P ODT. Further details may be obtained from either address. 3700(A)

ROBERTSON RESEARCH INTERNATIONAL LIMITED

SEDIMENTARY GEOLOGISTS

Robertson Research International Limited have vacancies in their head office in North Wales for experienced sedimentary geologists. Applicants should be familiar with the full range of sedimentological techniques relevant to carbonates and/or clastics. The position is challenging with career prospects, and will require the successful candidate to work on individual assignments and as a member of a team. Work is concerned with the study and interpretation of rocks from oil prospective areas. Preparation of technical reports is an integral part of the duties and experience in this field is required. Salary will be competitive and negotiable depending on age, experience and qualifications. The position is permanent and pensionable. The closing date for completed application forms is 31st December 1979.

Please apply to: (Quoting Ref. P/13-2) Mrs Janet Hedges, Robertson Research International Limited, 'Ty'n-y-Coed', Llanrhos, Llandudno, Gwynedd, LL30 1SA, Gwynedd. 3677(A)

UNIVERSITY OF GEZIRA/SUDAN

Applications are invited for the post of

Lecturer

in Weed Science in the Faculty of Science and Technology.

Applicants must have a higher degree and relevant teaching and research experience in the area of weed research and chemical weed control. The appointee is expected to be engaged primarily in research and postgraduate teaching, although duties may include limited teaching at the undergraduate level. The appointment will be for a fixed term of two years with possibility of renewal.

The salary will be determined according to experience. This position is endowed by CIBA-GEIGY. Children's education allowance, health insurance, holiday visit passages, family passages, free housing, and annual overseas leave will be provided.

Detailed applications (3 copies) with curriculum vitae and naming 3 referees to be sent to the Vice-Chancellor, University of Gezira, P.O. Box 20, WAD MEDANI, Sudan. General information and conditions of service or contract may be obtained from this address. W509(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF SOIL SCIENCE

Applications are invited for the post of

LECTURER

in the Department of Soil Science tenable from 1st January, 1980. Whilst research experience in the use of physical methods for the solution of soil, agronomic or environmental problems would be particularly appropriate for this post, applications from those with interests in the broader aspects of soil management and fertility will also be welcomed.

Salary will be at an appropriate point on the scale £4,333 to £8,992 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar (F.P.), The University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (three copies) together with the names and addresses of three referees, should be lodged not later than 26th November, 1979. Please quote reference N. 3678(A)

Nature Vol. 282 8 November 1979

CSIRO AUSTRALIA

Research Scientist

Division of Plant Industry Canberra

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees — 2400 of who are research and professional scientists — located in Divisions and Sections thoughout Australia.

Field: The management of agricultural systems.

General: The Divisions, with headquarters in Canberra, carries out research in a wide range of plant sciences fundamental to agricultural production. Its Agricultural Systems Programme is concerned with developing more efficient systems of farming in the temperate high-rainfall zone, through a combination of experiments and simulation techniques. Of particular interest are the problems associated with grazing systems based on improved pastures and their integration with crop production. The Division has an Experiment Station and Animal House facilities within easy reach of Canberra.

Duties: The appointee will participate, with a team trained in agronomy, animal nutrition and simulation techniques, in a programme aimed at increasing the efficiency of mixed farming systems. In particular, he/she will be expected to devise methods of overcoming problems that limit animal production in temperate areas of the high-rainfall zone. The research programme will aim at defining deficiencies in herbage against the background of seasonal animal requirements. Its emphasis will be on ways in which shortages can be met through supplements, pasture improvement and the grazing of crops and stubbles.

Qualifications: A PhD degree or equivalent qualifications and training in an appropriate field, together with demonstrable research ability. Experience in the use of techniques for studying the nutritional status of suckling and grazing animals, will be an advantage.

Salary: Research Scientist or Senior Research Scientist $A15,916 — $A23,122 pa.

Tenure: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 130/1459 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 7th December 1979.

Applications in U.S.A. and Canada should be sent to: — The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 3688(A)

Assistant Professor
Yale University
DEPARTMENT OF BIOLOGY

This Department invites applications for appointment beginning in academic year 1980-81 as Assistant Professor (level I or II) from persons with interests in molecular aspects of Genetics or Development. The appointee will be expected to contribute to a strong program of research and scholarship in Molecular Biology, and to participate in undergraduate and graduate teaching. Send curriculum vitae, resume of past and present research, and names of three referees by 1 January, 1980 to:

Chairman
Department of Biology
Yale University
New Haven, CT 06520

Yale University is an Equal Opportunity/Affirmative Action Employer
W518(A)

UNIVERSITY COLLEGE OF NORTH WALES
Bangor

CHAIR OF COMPUTER SCIENCE

Applications are invited for the post of

PROFESSOR OF COMPUTER SCIENCE

in the School of Mathematics and Computer Science.

The appointment will be from 1st October 1980 (or an earlier date to be arranged) and the salary will be in the professorial range.

Further particulars can be obtained from the Secretary and Registrar, University College of North Wales, Bangor, Gwynedd, LL57 2DG.

Ten copies of the application (one from overseas candidates) together with the names of three referees should be sent to reach the Secretary and Registrar by Friday, 14th December, 1979. 3680(A)

UNIVERSITY OF DUNDEE

Applications are invited for a newly established

CHAIR OF ZOOLOGY

in the Department of Biological Sciences. There is at present one other chair in the Department, the Boyd-Baxter Chair in Biology which is currently held by Professor W D P Stewart, FRS.

Further particulars are available from The Secretary, The University, Dundee DD1 4HN, with whom applications (ten copies or, if posted overseas one copy) should be lodged by November 22nd, 1979. Please quote reference EST/86/79J. 3576(A)

UNIVERSITY OF CAMBRIDGE

CAVENDISH LABORATORY

Applications are invited from suitably qualified physicists, chemists or metallurgists for a post doctoral research post jointly funded by ICI and the SRC under the Collaborative Award Scheme. The work is concerned with the structure and composition of very highly dispersed supported metal catalysts of enhanced activity and will provide an insight into the application of advanced research techniques to systems of direct industrial interest. Experience in electron microscopy and related techniques is required.

The appointment will be for two years, based in Cambridge but with about 10% of the period at the Corporate Laboratory of ICI at Runcorn. Salary is in the range £4333 — £5199 per annum. Applications should be addressed to the Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE. 3673(A)

KINGSTON POLYTECHNIC

SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

RESEARCH ASSISTANT IN HETEROCYCLIC CHEMISTRY

The work, supervised by Dr D T Hurst, will be concerned with the synthesis of pyrimidines and related compounds, the electrophilic substitution of pyrimidines, and studies of physico-chemical and biological properties of such compounds.

Applicants should have an honours degree, GradRIC or equivalent qualification, and if successful, will be expected to register for a higher degree.

Salary range £3,174 to £3,462 inclusive. Further details and application forms from Dept AO, Academic Registry, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. 3696(A)

DEPARTMENT AND BIOPH...

The Department is undertaking ... ch for a relatively senior biophysicist (associate to full professor level) with rather broad interests and expertise in the general area of spectroscopy with possible, but not necessary, emphasis on nuclear magnetic resonance.

Prospective candidates should send their curriculum vitae and bibliography to Dr J Kent Blasie, Chairman, Search Committee, Department of Chemistry, 550 Chemistry Labs/D5, University of Pennsylvania, Philadelphia, Pa. 19104.

The University is an equal opportunity/affirmative action employer. W506(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF PHYSICS & ASTRONOMY

POSTDOCTORAL RESEARCH ASSISTANT IN HIGH ENERGY PHYSICS

Applications invited for a post available immediately for a Physicist to work with a team participating in the experimental programme of the SPS accelerator at CERN, Geneva. Appointment initially in Geneva, participating in the design, installation, operation and data analysis of a recently approved hybrid emulsion-electronic experiment to search for B particles.

Salary in range £4,333 — £5,488 plus £740 London Allowance. In Geneva fares, housing and additional allowances will be provided.

Candidates should send details of their qualifications and experience, together with the names of two referees, to Dr B C Duff, Dept. of Physics and Astronomy, University College London, Gower St. London WC1E 6BT as soon as possible. 3701(A)

THE HATFIELD POLYTECHNIC

SCHOOL OF NATURAL SCIENCES

SENIOR LECTURER/ LECTURER II IN BIOCHEMISTRY

Applications are invited from suitably qualified candidates for a vacancy in the field of either Toxicology or Molecular Biology preferably including eukaryotic systems.

Candidates should possess an honours degree or equivalent qualification in Biochemistry or a related area, together with a postgraduate degree or substantial research experience. Industrial or other applied experience would be a decided advantage and applicants will be expected to be thoroughly conversant with the modern methodology and techniques involved in their field.

Salary scale: Lecturer II £4470 — £7149 + £72 supplement + £159 local weighting.

Senior Lecturer £6597 — £8253 + £72 supplement + £159 local weighting.

Application form and further details are obtainable from the Staffing Office, The Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts or telephone Hatfield 68100 extension 309. Please quote reference: 298. Closing date: 23 November 1979. 3656(A)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY
GRADUATE RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work with Dr T ap Rees and Dr R A Leigh on the storage, metabolism and mobilisation of sucrose by vacuoles isolated from higher plants. Applicants should have a degree in biochemistry, botany, or a related subject. The appointment, which is financed by the SRC, will be for a maximum period of 18 months and will commence as soon as is practicable.

The starting salary will be £3,775. The successful candidate will not be able to register for a higher degree.

Applications, naming two referees, should be sent to Dr T ap Rees, University of Cambridge, Botany School, Downing Street, Cambridge CB2 3EA. 3697(A)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF COMPUTER SCIENCE
PROFESSOR OF COMPUTER SCIENCE

Applications are invited for a second Professorship in the Department of Computer Science. To preserve the balance of interest in the Department, candidates with a knowledge either of Data Bases or the Theory of Computer Science are particularly encouraged to apply.

The post will be remunerated within the Professorial range for universities with USS benefits.

Application forms and further particulars (quoting 38/79) can be obtained from the Registrar, University of Strathclyde, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by 15 December, 1979. 3711(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF METALLURGY & MATERIALS SCIENCE
POST-DOCTORAL SENIOR RESEARCH ASSISTANT/RESEARCH ASSOCIATE

Applications are invited for the above post which is supported by the UKAEA Harwell and is tenable for two years. The area of research is intergranular fracture in iron based alloys and the objective will be to develop a fundamental understanding of the factors governing segregation of solutes to grain boundaries and their role in promoting grain boundary embrittlement.

It is expected that studies will be made of the thermodynamics of segregation and how this is related to composition and microstructure. The effects of segregated solutes on the work of fracture at grain boundaries will also fall within the scope of the work.

The appointee will be expected to spend periods at Liverpool and Harwell and will have access to surface analytical instruments, electron microscopes equipped for micro analysis and sophisticated mechanical testing equipment.

Initial salary will be on the scale £4,333 - £5,777 per annum.

Application forms and further particulars may be obtained from The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, by whom completed forms should be received not later than 30th November, 1979. Quote ref. RV/830/N. 3666(A)

RESPIRATORY PHYSIOLOGY TRAINING PROGRAM
Funded by NHLBI
POST-DOCTORAL TRAINEESHIPS

available for one to three years. Areas of research include gas transport, red blood cells, microcirculation, neonatal physiology, aortic body and carotid body chemoreceptors, lung metabolism, lung cells, (type II and pleural) smooth muscle, lung innervation, tissue oxygenation, mitochondrial function and control of respiration.

Write to Education Officer, Respiratory Physiology Group, Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104. W517(A)

UNIVERSITY OF QUEENSLAND
Australia
SENIOR TUTOR IN ZOOLOGY
(2 positions)

Applicants should have a higher degree or honours with special interests in Invertebrate Zoology and/or Cellular Biology.

Salary: $A14,041 to $A16,085 p.a. Other benefits: Superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date 22 November, 1979. 3684(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF MOLECULAR BIOLOGY
POSTDOCTORAL RESEARCH FELLOW

required as soon as possible to work on the molecular relationships of plasmids from strains of *Pseudomonas* and of enterobacteria. The methods currently being used include genetic manipulation *in vitro*, DNA-DNA hybridisation and electron microscopy of heteroduplexes.

The position is for two years. Further information from Dr P M A Broda (tel: 031-667 1081, ext. 2886) to whom applications should be sent with the names of referees and details of experience. Please quote Reference 5063. 3669(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF ZOOLOGY
HISTOLOGY TECHNICIAN (GRADE 5)

Candidates should have HNC or equivalent appropriate qualification and several years laboratory experience. The successful applicant will be responsible for preparing histological material for the teaching and research needs of the Department. Salary will be within the range £3,700 to £4,320 p.a.

Application forms may be obtained from The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote reference RV/837/N. 3692(A)

ASSISTANTSHIPS

WELLCOME SUPPORTED RESEARCH ASSISTANTSHIPS IN IMMUNOLOGY
UNIVERSITY OF CAMBRIDGE
Applicants are invited for
TWO RESEARCH ASSISTANTSHIPS

to participate in a research programme with Professor R.R.A. Coombs on sensitive methods for detecting antibodies and surface antigens.

One research assistant should be experienced in immuno-chemical purification, fractionation procedures and specificity control. The other should have experience in separation of lymphocyte subpopulations, lymphocyte stimulation, rosetting procedures and cell culture.

It is anticipated that the project will become operative on January 1st, 1980. Applicants should be under 28 at this date, possess a science degree, preferably also a PhD, and have distinctive laboratory experience. Salary up to £5,488 at 28.

Enquiries to Professor R.R.A. Coombs, Division of Immunology, Laboratories Block, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, and applications to the Laboratory Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. 3715(P)

UNIVERSITY OF GLASGOW
DEPARTMENT OF NATURAL PHILOSOPHY
POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for an SRC funded post-doctoral research assistantship tenable for two years, to work with Dr E W Laing on computer simulation studies of compression of plasma to very high densities, in connection with Heavy Ion Fusion. Commencement date by arrangement, but not later than 1 February, 1980.

Initial salary in the range £4,333 to £4,910 (under review) on Range 1A of the scales for Research & Analogous Staff, plus superannuation.

Applications naming two referees should be sent by 1st December, 1979 to Dr E W Laing, Department of Natural Philosophy, University of Glasgow, Glasgow G12 8QQ, from whom further particulars may be obtained.

In reply please quote Ref No 4538M. 3693(P)

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COURSES

EMBO
Advanced Laboratory Course on "Molecular Biology of Membranes"
co-sponsored by FEBS & SKMB
14-27 September 1980
Bio-Center, Basel, Switzerland

The course will include review lectures, specialized lectures and laboratory experiments. Main topics to be covered will include the physical chemistry of membranes, membrane reconstitution and analysis of membranes by biochemical, genetic and immunological methods.

Laboratory space will be available for 24 students. The course fee will include Swiss Francs 100 for registration and approximately Swiss Francs 500 for accommodation and food. Fellowships will be available. Applicants will be selected on a world-wide basis.

Application forms can be obtained from the organizers. Deadline for application is April 1, 1980. Organizers: G. Schatz and J Seelig, Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. Tel. 061/25 38 80. W520(D)

UNIVERSITY OF LEICESTER
DEPARTMENT OF CHEMISTRY AND SCHOOL OF EDUCATION
Ph.D. in Chemistry with Graduate Certificate of Education

Applications are invited from persons holding, or expecting to be awarded during 1980, a degree with First or Second Class Honours (Upper division), or equivalent qualifications, in Chemistry, Combined Sciences, Chemical Engineering, Physics etc., who wish to pursue a 3-year course of research and teacher training.

The course may attract a limited number of Science Research Council quota awards, principally for new graduates, but candidates from industry or teaching may be eligible to apply for awards under other S.R.C. schemes.

For further information and application forms please write to Professor R D Peacock, Department of Chemistry, University of Leicester, LE1 7RH.

The closing date for applications is January 31st 1980. 3658(D)

FELLOWSHIPS

UNIVERSITY OF OXFORD
DYSON PERRINS LABORATORY

Applications are invited from organic chemists for a postdoctoral fellowship concerned with the applications of organometallic catalysts to asymmetric synthesis.

The appointment will be for a period of up to two years in Research Assistant Grade 1A, on a salary up to £4910, with membership of USS.

Candidates should write as soon as possible to Dr J M Brown, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY enclosing a full curriculum vitae and quoting the names of two referees. The starting date is flexible and final-year Ph.D. candidates will be considered. 3708(E)

FELLOWSHIPS and STUDENTSHIPS

Travelling Research Fellowships to Europe 1980-81

Applications are invited from British Research workers for the award of Wellcome-European Research Fellowships for 1980-81. These awards include the Trustees' special fellowships to Denmark, Norway, Sweden, Finland and Hungary.

The object of these fellowships is to encourage working visits to countries in Western and Eastern Europe by investigators in any branches of the natural and clinical sciences which have a bearing upon human or animal medicine. Candidates whose research is related to clinical problems will be given preference. These fellowships, which are normally for one year, are intended for *graduates from British Universities*, who already have some post-doctoral research experience. Stipends, according to age and experience, are within the range of £4,622 - £7,815 per annum. A special cost of living allowance applicable to the country in which the candidate wishes to work is given where necessary. Travelling and some incidental expenses are provided in addition.

Candidates must make their own arrangements with the department in which they propose to work, and written evidence must be submitted confirming their arrangements.

Application forms may be obtained from the Grants Section, The Wellcome Trust, 1 Park Square West, London NW1 4LJ. Telephone: 01-486 4902. Completed application forms must be returned by 31st December 1979.

The Wellcome Trust

3698(E)

THE ROYAL SOCIETY
E.P.A. Cephalosporin Fund SENIOR RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for the E.P.A. Cephalosporin Fund Senior Research Fellowship, endowed by the Trustees of the E.P.A. Cephalosporin Fund to provide a research fellowship in the medical, biological and chemical sciences. On this occasion applications are restricted to those working in the field of biological chemistry. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from 1 October 1980 (or another date to be arranged) and may be renewable for one further five-year period. Applicants should preferably be under 40 on 1 October 1980. The stipend scale will be £9138 × £300 to £10 518 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and returned by 15 January 1980.

3699(E)

UNIVERSITY OF KENT AT CANTERBURY
APPOINTMENTS IN SPACE SCIENCES IN THE ELECTRONICS LABORATORY

Applications are invited for the following research posts tenable from 1 December, 1979 or as early as possible thereafter and financed by Science Research Council grants under the direction of Dr J.A.M McDonnell:

(1) A 3-year Post-Doctoral Fellowship for the development of instrumentation for the detection and analysis of cosmic dust on future space missions. Experiments for the joint ESA/NASA Comet Mission, Recoverable Space Shuttle Packages, Lunar Orbiters and Refurbishable Satellite Systems are planned. Relevant research experience in physics, astronomy, space research or instrumentation is desirable. Salary Scale: £4333 — £7521;

(2) A 2-year Post-Doctoral Fellowship for sensor calibration, mission development and data analysis in connection with the International or Solar Polar (Out of Ecliptic) Mission in collaboration with the Max Planck Institute, Heidelberg, and the Space Shuttle Recoverable Long Duration Exposure Facility. Suitable post-doctoral experience in space projects is essential for this post. Salary Scale: £4333 — £7521.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH to whom completed applications should be returned by 30 November 1979. Please quote ref. A44/79.

3716(E)

THE ROYAL SOCIETY
EUROPEAN SCIENCE EXCHANGE PROGRAMME FELLOWSHIPS

Applications are invited by the Council of the Royal Society for a research fellowship in the European Science Exchange Programme tenable in a laboratory in Austria, Belgium, Denmark, Finland, France, Federal Republic of Germany, Greece, Republic of Ireland, Italy, the Netherlands, Norway, Portugal, Spain, Sweden or Switzerland. Candidates who must be of postdoctoral status by the time the proposed visit commences should have made preliminary arrangements for acceptance at the laboratory of their choice. Preference is given to newly or recently postdoctoral applicants, but candidates holding permanent appointments in the United Kingdom are also eligible to apply; in such cases the award will take into account any continuing salary which the candidate will receive.

Fellowship awards are normally made for 12 months, although applications for less than a full academic year but not less than 6 months will also be considered. Two-year fellowships may be awarded to a few outstanding candidates where the project merits this. Awards are based on the United Kingdom university lecturer rates plus local and married allowances.

The closing dates for applications are 12 January and 10 May 1980 and those interested should write for further details and an application form to The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

3681(F)

POSTDOCTORAL POSITION

To work in transport of organic solutes in mammalian systems, membrane proteins and reconstitution of transport systems.

Please write to Dr R M Johnstone, Department of Biochemistry, McGill University, 3655 Drummond St., Montreal, Quebec, Canada, H3G 1Y6.

W504(E)

UNIVERSITY OF ABERDEEN
MILNER STUDENTSHIP

Applications are invited from candidates for this research studentship in the Department of Pathology tenable for one year in the first instance, with the possibility of extension for two further years. The research subject is a study of endothelial cell growth and reactions by tissue culture, autoradiographic and ultrastructural methods. Applicants should possess a good honours degree in some aspect of biology, preferably cell biology, pharmacology or physiology. It is expected that the appointee will register as a PhD student.

The value of the studentship and associated allowances will be broadly in line with those offered by the Research Councils i.e. in 1979-80 £1820 per annum plus University tuition fees.

Further particulars and information can be obtained from Professor A L Stalker, Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, AB9 2ZD. Tel: 0224 681818, extn. 2411.

Applications, with the names of two referees, should be lodged with the Secretary, The University, Aberdeen, by 30th November, 1979.

3675(F)

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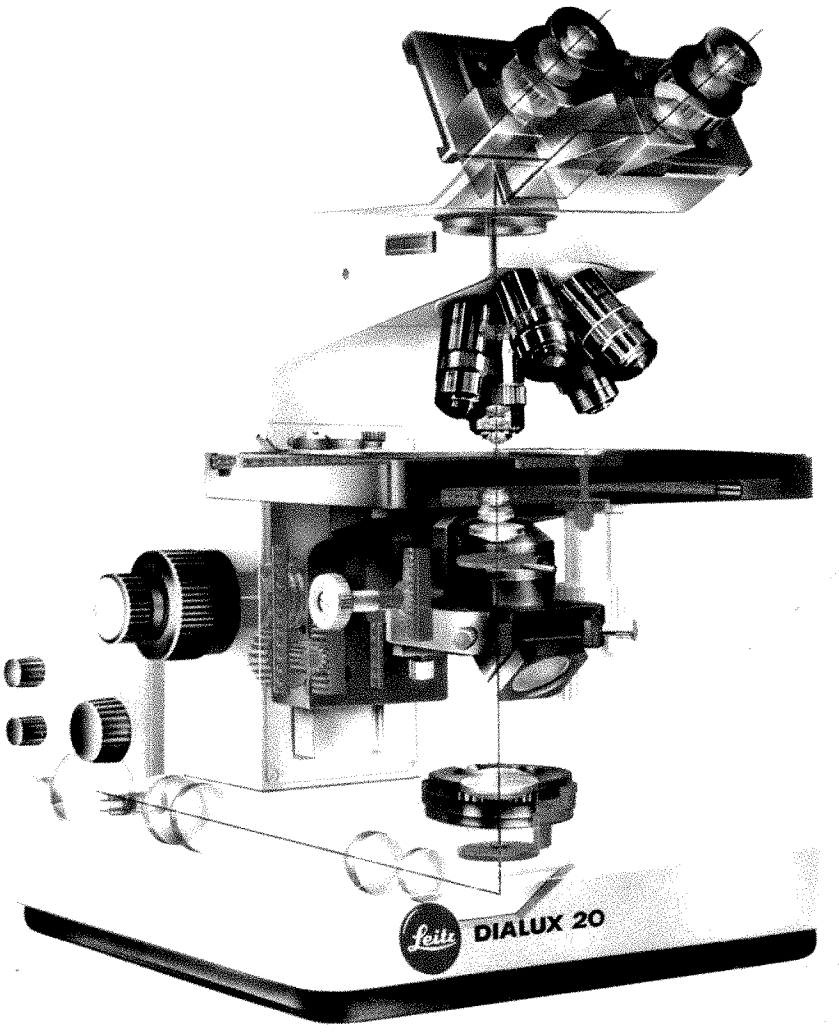
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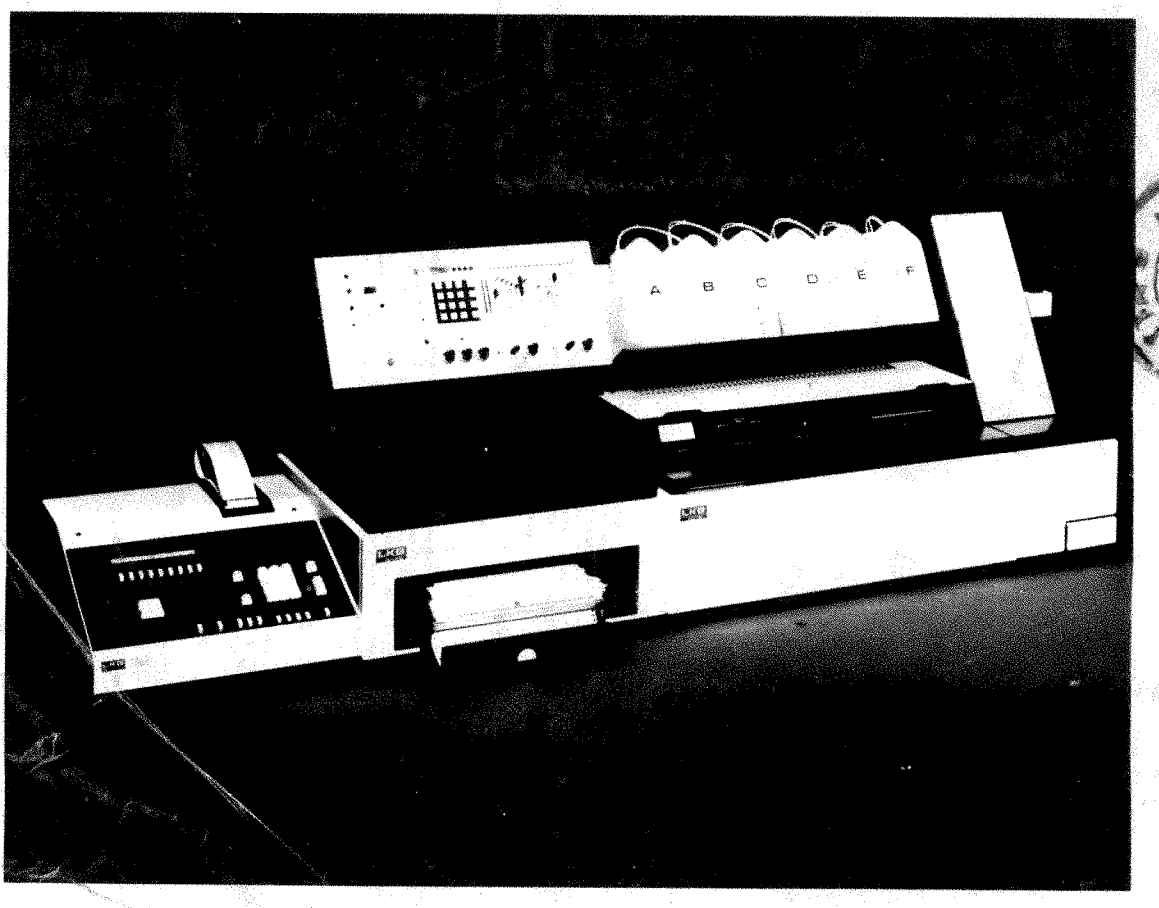
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nature

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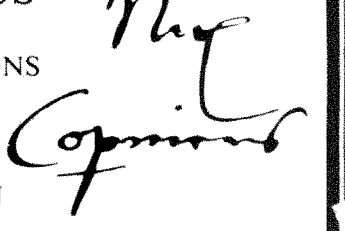
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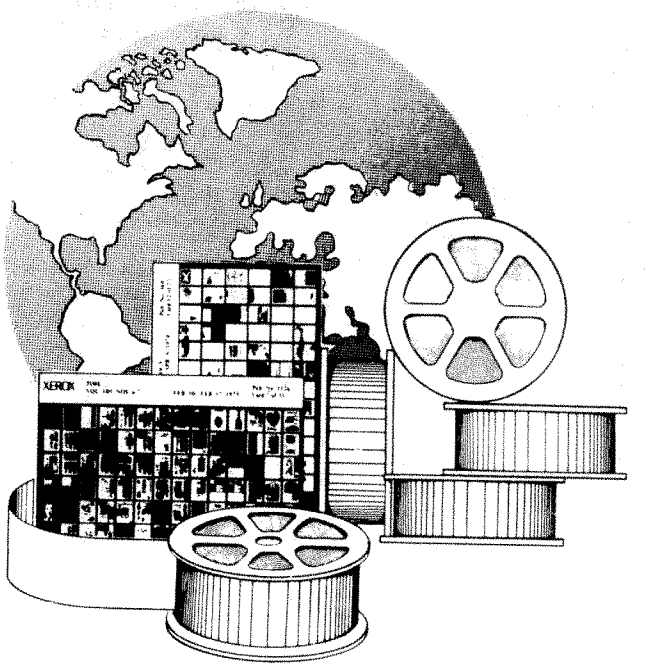
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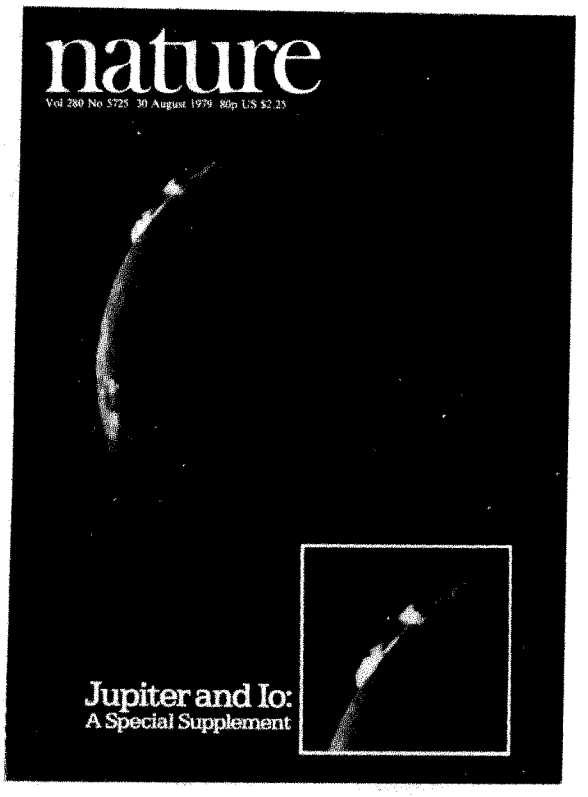
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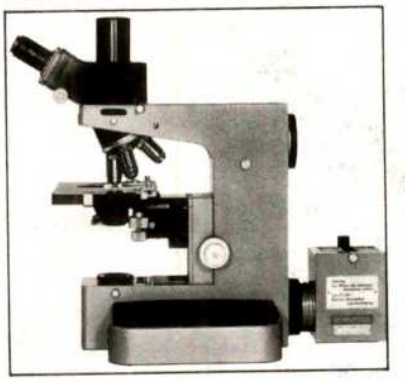
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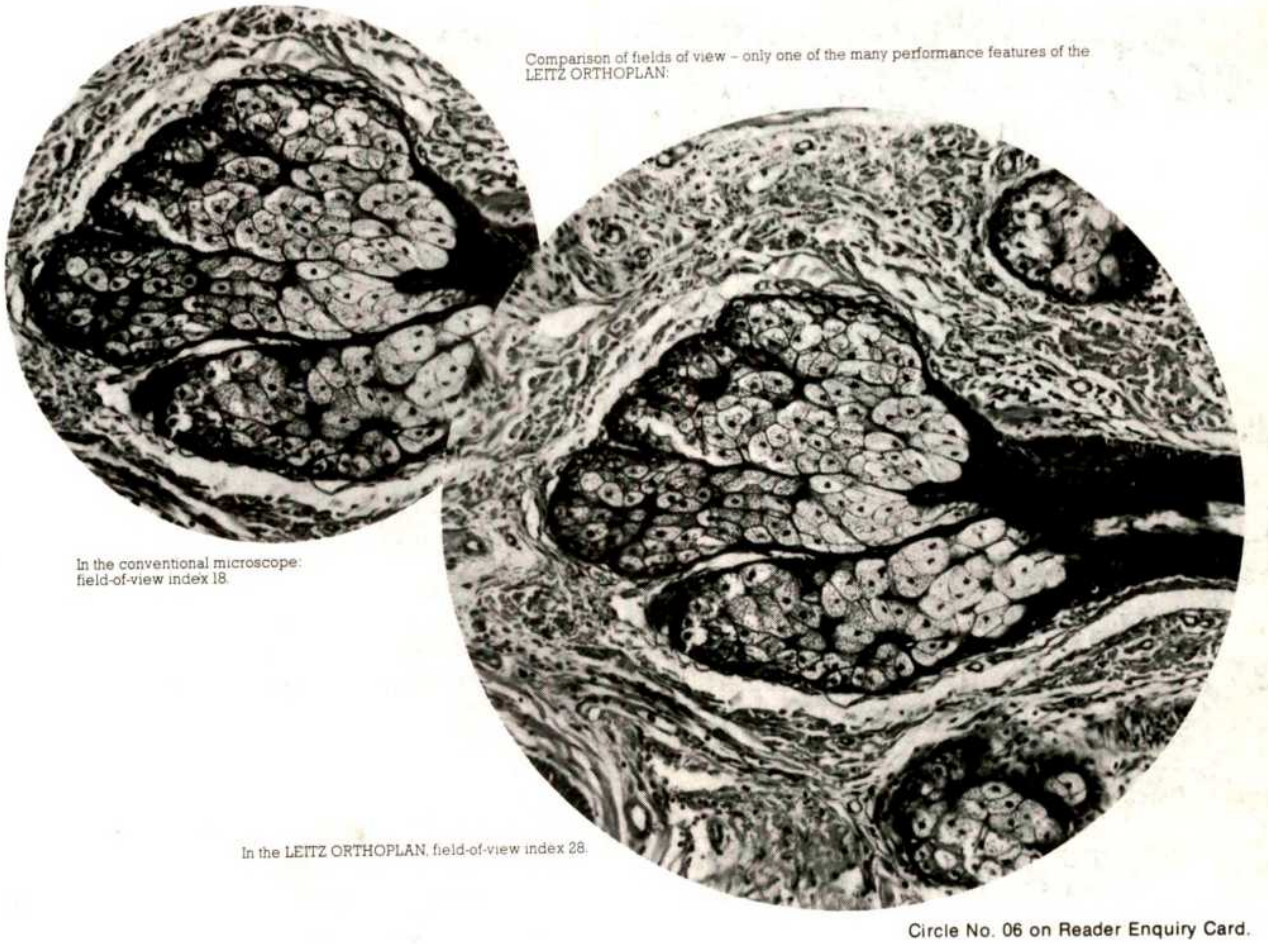
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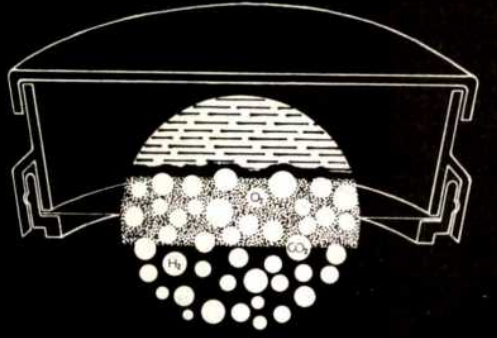


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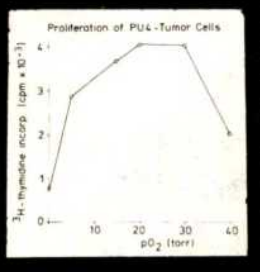
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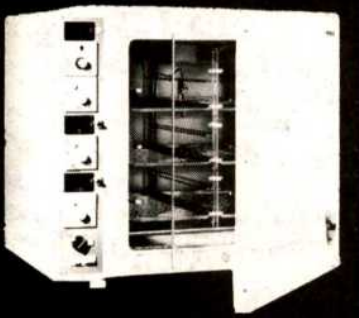
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Last chance for UNCSTD?

THERE have been two main reactions to the outcome of the United Nations Conference on Science and Technology for Development, which took place in Vienna in August. Some delegations returned home convinced that the conference had been a success; they support this claim by emphasising the amount of common ground established between developed and developing countries, and the general agreement on directions for future cooperation. Others, perhaps greater in number, returned feeling the conference had been a failure — but a qualified one. It had not resulted in any major shift in research towards Third World needs, or any particular clarification of what these needs are — but a framework had been agreed for pursuing both of these objectives.

Which of these two interpretations eventually prevails will depend on the outcome of the debates scheduled to take place next week at the UN General Assembly in New York. The Assembly's second committee will be discussing both the results of the Vienna conference, and the specific ways in which actions agreed by the conference should be carried out. Unfortunately many points of contention still remain, and it would be a tragedy if the short-term perspective engendered by the economic problems of the developed countries, as well as continued in-fighting within the UN system over future roles and responsibilities, placed in jeopardy the fragile achievements of Vienna.

There has already been some progress since the conference ended. The Director General for Development and International Economic Cooperation has prepared a detailed outline for the new intergovernmental committee (IGC) to oversee 'science, technology and development' issues. The Advisory Committee on the Application of Science and Technology to Development (ACAST), which despite recent criticism has considerable past achievement to its name, will be absorbed into a new body to advise the IGC. And the United Nations Development Programme, charged with administering the interim fund which the conference agreed should be set up on voluntary contributions totalling at least $250 million for 1980 and 1981, has produced a business-like prospectus outlining how it will carry out this role.

But it would be wrong to think that next week's meeting will be anything like plain sailing. There are several controversial issues still to be decided, in particular the division of responsibilities within the UN structure for science and technology activities, and levels of appointment and staffing to service the new committee. The Group of 77, which has been negotiating for the developing countries, is expected to present its proposals to the second committee outlining its veiws on these matters. And given their importance to both developed and developing countries — the Vienna conference, for example, was unable to agree on the specific role which the IGC should play with respect to the interim fund — heated discussion seems inevitable.

In terms of extra financial transfers, the situation looks even bleaker than it did in Vienna. Britain has just announced new foreign aid cuts which make it even more unlikely that it will be prepared to contribute to the interm fund when a pledging conference is held next February. The French position on an apparent commitment to increased UNDP contributions remains ambiguous. And Canada is reported to be having second thoughts about its proposal to shift funds within its aid budget to support joint research projects between Canadian and Third World research workers: Canada's International Development Research Centre, which had been expected to receive some of the new funds, is having its expansion plans closely scrutinised.

Perhaps the most alarming news comes from the United States, where the new Institute for Scientific and Technical Cooperation proposed by President Carter and offered as a centrepiece of the US presentation at Vienna is in serious defficulties in Congress. The Senate has refused to provide any funds for the institute's operation. Despite pressure from the House of Representatives, legislators seem determined to turn the ISTC into a sacrificial lamb on the altar of reduced public spending and federal bureaucracy. And its fortunes do not augur well for Congressional response to the $25 million request which President Carter is expected to make in January for the UNDP interim fund — already the source of considerable controversy in the State Department — over where the money is to come from.

Yet if there was one message to come through from UNCSTD, it was that helping to promote scientific and technological activities in the Third World is not a question of handing our blank welfare cheques — it is an essential prerequisite for a flourishing global economy.

The tighter the purse strings, the more difficult this will be to achieve. And ironically the more important it becomes to ensure that international mechanisms such as the UNDP and the new IGC can be made to work fairly and effectively. □

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US scientists double estimates of ozone depletion rate

A COMMITTEE of the US National Academy of Sciences has calculated that the worldwide use of halocarbons may be depleting the ozone content of the stratosphere twice as fast as was predicted three years ago. A report published by the academy in 1976, calculating a depletion rate leading to an eventual 7.5% reduction in ozone, became the basis for a ban in the US, implemented at the end of last year, on all non-essential uses of chlorofluorocarbons, particularly those used as aerosol propellants.

Using improved computer models and better atmospheric and laboratory measurements, the panel on stratospheric chemistry and transport of the Academy's National Research Council now estimates the most probable eventual depletion as 16.5%.

Furthermore, using a model that assumes "an increasing release rate in other industrial countries" — rather than a release rate fixed at the 1977 level, the panel says it is "highly probable" that the eventual steady state reached would involve a 30% ozone reduction — and that this could be as high as 56.7%.

The panel points out that many uncertainties still surround such calculations. Since the release of earlier reports indicating the possible danger from fluorocarbon use, however, it points out that there has been a considerable improvement in both computer models and the quality of atmospheric measurements used to check their validity.

These activities have not altered the principal conclusion reached in the previous reports that continued release of halocarbons into the atmosphere will result in a significant decrease in the amount of stratospheric ozone," the report says.

Based on continued emission at the 1977 level the report says its own best estimate of the eventual ozone depletion is 16.5%. It adds that its estimate of uncertainty is such that there is a 95% probability that the true value of the ozone depletion lies between 5% and 28%. Ozone reduction to half the eventual level — to 8% — would occur in about 30 years, the report adds. □

Herbicide claimed responsible for birth defects

A US chemical trade union claimed last week that the exposure of male workers during production of the herbicide Oryzalin may have resulted in genetic damage to the children that they subsequently conceived.

According to the International Chemical Workers Union, of the four children born to men working in the plant in Rensselaer, New York State, over the period January 1975 to June 1976 during which the herbicide was being produced, three were born with heart deformities — two later dying — and one died from pneumonia two months later. There was also one miscarriage.

Oryzalin is the trade name for the chemical 3,5,-dinitro-N(4), N(4)-dipropylsulfanilamide. It is marketed in the US by Eli Lilly and co, which has denied that there is any evidence connecting the worker's exposure to the birth defects.

The company points out that no birth defects were indicated in laboratory tests carried out with rats and rabbits before the herbicide was approved by the Environmental Protection Agency in 1975. Nor was any evidence discovered after the question of a link to the human birth defects first raised in 1976. However, the assistant administrator of the EPA, Mr Steven Jellinek, said that the Agency was "very concerned" about the union's charges. Although the EPA did not know of any "scientifically established link" between exposure to Oryzalin and these health problems, the union's information indicated that there might well be some hazard, either from the herbicide or from some other substance to which the workers were exposed.

The union has asked the federal government to ban the production and use of Oryzalin, which is used on soybeans for weed control. "We are taking these actions to alert the public to the potentially devastating effects to future generations from exposure to this chemical" said union President Frank D Martino.

Mr Jellinke said that although no problem had shown up in the animal tests, the human experience indicating possible genetic problems with workers exposed to the herbicide "does raise questions". He said that the EPA will set up a team with the Occupational Safety and Health Administration and the National Institute of Occupational Safety and Health to study the effects of the herbicide, and that the report should be ready "within weeks".

Last week the EPA suspended all use of the pesticide DBCP while it decides whether to impose a permanent ban on the chemical, which has been linked both to cancer, and to sterility in male workers involved with its production. The administrator of the EPA, Mr Doug Costle, has ordered all applications of DBCP — most commonly used on fruit trees and soybeans — to be halted except in Hawaiian pineapple groves.

David Dickson

US sifts ex-Nazi scientists for possible war criminals

THE US Department of Justice, stepping up its search for Nazi war criminals who may now be resident in the United States, is investigating whether any were included in the 500 scientists and engineers who were brought over from Germany at the end of the second World War to work on weapons research.

No details have been given of the names of individuals under investigation. However, Justice Department officials confirmed last week that some of the scientists and engineers involved in what was known as "operation paperclip" are among the department's list of 200 to 250 suspected war criminals.

Under the programme, which operated from 1945 to 1955, over 3000 German and Austrian scientits who had worked for the Nazis were checked by US authorities to discover whether their skills could be of value to the US military efforts — and thus might also be of value to the Soviet Union.

Over 500 were subsequently brought to the US under a programme approved by President Truman, despite strong opposition from scientists such as Albert Einstein.

Since it was felt that it was in the interests of national security to bring them to the US, the scientists and engineers were admitted without having to pass the usual immigration and visa checks, even though some were known to have been members of the Storm Troopers, and the League of National Socialist German Physicians.

Perhaps the best known of those admitted were the members of the team which worked on guided missiles under Dr Werner von Braun, and were largely responsible for launching the first US satellite in 1958. However, the US also brought over experts from a variety of other disciplines, such as three scientists who had worked on the German chemical weapons programme, who went to work for the Army's Chemical Warfare Division.

Mr Martin Mendelsohn, deputy director of the Justice Department's newly-created office to investigate Nazi war criminals, said last week that membership of a Nazi organisation was not necessarily sufficient to invoke charges, which required evidence of war crimes. He confirmed that his office is now examining the files of all those brought to the US under operation paperclip to see if any cases should be pursued further.

A typical case demanding attention, according to a staff member of the Senate committee which is pushing the Administration for action on war criminals, is that of a former concentration camp medical officer who came to the US to work on a variety of aviation medicine projects. □

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Rasmussen vindicated?

The Three Mile Island accident might have been prevented if more attention had been paid to a report on nuclear risk, but its message was clouded by disputes. **David Dickson** reports

ONE unexpected spin-off from the accident at the Three Mile Island nuclear plant last March has been a shift in attitude towards a report on the safety of nuclear reactors published in 1975 — the much-criticised Rasmussen Report.

Known officially as Wash 1400, and commissioned by the Nuclear Regulatory Commission, the report is usually referred to under the name of its principal author Dr Norman Rasmussen, now professor of nuclear engineering at the Massachusetts Institute of Technology.

When the report first appeared, the nuclear industry made much of the report's conclusion that the level of risk associated with nuclear power was relatively low compared to other energy sources. But it also received a barrage of criticism, in particular over the validity of broad conclusions drawn from limited data.

Much of this criticism was subsequently endorsed by a review committee established by the NRC, which repudiated some key sections. But the report of the President's Commission on the TMI accident, chaired by Dr John Kemeny and released two weeks ago, points out that the sequence of events leading to the accident supports Rasmussen's general analysis — with the implication that if more attention had been paid to the positive aspects of the report, the accident might have been avoided.

Professor Rasmussen is unapologetic about the report. He accepts that some aspects of the accident, in particular the extent of its psychological impact, were not predicted. But he points out that the report emphasises the dangers of concentrating safety planning on single major equipment failures, rather than on an accumulation of relatively minor failures compounded by operator error. "If anything, we were too conservative. We said that if the temperatures in the core went as high as they did, then we did not know if it would melt or not, but said it might have done. In the event the core did not melt, though a few more human errors would have caused it to do so."

Part of the problem is that there are, in a sense, two Rasmussen reports. The first is a detailed application of fault-tree/event-tree analysis to equipment failures in nuclear power plants. Even critics acknowledge this as an important contribution towards understanding risks. And the NRC review, carried out by a committee under Professor H W Lewis of the University of California, stated that the methodology developed "both can and should be more widely used by the NRC".

The second version of the report, however, is the one that was presented through the news media as carrying the principal message that nuclear power is basically safe. This conclusion, for example, helped persuade Congress to accept the Price-Anderson Act, limiting the liability of power companies in the case of nuclear accidents.

Professor Rasmussen is himself critical of those who over-sell the safety of nuclear power. "In the past there was a general tendency of public relations people to under-estimate the intelligence of the public, leading to the feeling that it was sufficient to issue warm pacifying statements rather than realistically portraying the risks and benefits of nuclear power. As the facts have evolved, however, the nuclear supporters have lost credibility, and many of their arguments are no longer believed."

Credibility has been at the heart of arguments over the Rasmussen Report itself. The Lewis Committee, following the lead of groups such as the Union of Concerned Scientists, criticised in particular the error bounds placed on the probabilities of various accident sequences.

They argued that these bounds were greatly understated, partly because of an inadequate data base, and partly because of an inability to quantify "common cause" failures, such as fire and earthquakes.

Professor Rasmussen defends his judgment. "I disagree with the Lewis Committee about margins of error. They found that the uncertainty was understated. I say that it might be understated in the safe direction, but I do not think it was greatly understated in the unsafe direction, because we already have 600 plant years of operating experience, and we have not melted a core. This gives an upper bound which is 30 times higher than ours".

It was the debates over the limits of uncertainty that led to the widest public criticism of the report. The NRC, for example, earlier this year issued a statement emphasising that as a result of its review "It does not regard as reliable the reactor safety study's numerical estimate of the overall risk of reactor accident."

Ironically, the vulnerability of the probability analysis seems to have resulted in that part of the report which was supported by the Lewis Review not getting the attention that it deserved.

A report prepared by the technical staff of the Kemeny Commission says that Wash 1400 contained three important messages. These involved the expected frequency of accidents (the TMI accident was well within the predicted range), methods for improving reactor safety through, for example, the use of fault-tree analysis to identify weak links, and the most likely types of accidents.



Rasmussen: unapologetic about the report

"Perhaps it is a fault of the report that these messages were not emphasised, because the conclusion most often associated with Wash 1400 — reactors are safe — receives the primary emphasis in the report. Perhaps it was the fault of the NRC that more effort was dedicated to criticising Wash 1400 than was applied to understanding its messages," the technical report (which does not necessarily reflect the Commission's views) says.

Rasmussen's critics argue that the construction of the report made it directly vulnerable to lop-sided interpretation. According to Dr Frank von Hippel of Princeton University, many scientists who read the executive summary of the report — also criticised by the Lewis Committee — "were infuriated by its Madison Avenue tone."

"If you follow the report backwards, from the appendices to the report itself to the executive summary, you find that at each level the qualifications and uncertainties of the previous level are dropped, so that what comes out is misleading because of what is omitted," he says.

Professor Rasmussen accepts some of the Lewis criticisms — but repeats that the TMI accident underlines the report's main conclusions. "The anti-nuclear people were able to use the accident to their advantage. But you can draw some satisfaction for both sides. Utility operators are now more prepared to listen to suggestions about safety than they were before, and on both the regulatory and the operating side, the event will lead to substantially reduced risk."

The accident had done nothing to change his support for nuclear power, a position now frequently expressed on public platforms. "Every new technology, when it was poorly understood, has attracted its doomsday prophets. Mostly this opposition has died away, and I suspect that nuclear power will probably go the same way. If we do not go on building nuclear plants, we will soon have major problems with the reliability of our power supply." □

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Swedish firm in recombinant DNA storm

RECOMBINANT-DNA research in private firms in Sweden has got off to a very shaky start. The only firm to have applied to use the technique is threatening to move abroad if permission to go ahead is not given.

The firm concerned, KabiGen AB, is half owned by the state pharmaceutical company Kabi Vitrum, and half by private interests. It was set up in 1968 to investigate new sources of raw materials for Kabi Vitrum and is currently researching the production of human growth hormone by recombinant DNA techniques. Recently, the firm applied to the Hybrid-DNA Committee for permission to go ahead with further development. Permission was given to carry out the work P3/EK1 or P2/EK2 conditions under the revised NIH guidelines.

Kabigen already had a virology laboratory nominally between P3 and P4 standards; but as there were small parts of it which could collect dust, the firm announced that it would rebuild it. But an article in a Stockholm daily paper, written by the chairman of the Centre Party organisation in the same area as KabiGen, was very critical of the firm's plans to build the laboratory, especially as it is in a densely-populated part of Stockholm, and demanded that nothing be done until the whole issue had been debated publicly.

KabiGen's director, Dr Bertil Aberg, said that the firm's existence hinged on the laboratory being built and that the research would be moved abroad if the local health and building authorities did not approve the proposed changes and give permission for recombinant-DNA work to be done there.

The Centre Party — the carrier of the Swedish anti-nuclear banner — drew a parallel between the two issues, saying that unless there is a public debate now, recombinant-DNA research would become the nuclear debate of the 1980s. But the Party had not checked its facts. While claiming that KabiGen was proposing to build a "high risk labortory . . . without counterpart in Sweden", it was evidently unaware that there are already 25 P3 laboratories within a five-mile radius of Stockholm, handling dangerous pathogens in the State Bacteriological Institute and in local hospitals. It is doubtful, too, whether making minor alterations in the existing laboratory could be called "building". In spite of this, Dr Aberg seems to regret his initial openness. "KabiGen is the only industry using recombinant-DNA techniques", he told *Nature*. "Universities are doing a great deal of work with them, but they are very wisely keeping quiet about it."

However, this is not strictly true. All universities and private industries have agreed to inform the Hybrid-DNA Committee, set up in 1975 under the Natural Sciences Research Council, of any recombinant-DNA research they want to do. The committee then advises them about security levels. However this system will shortly be changed, if a bill proposed by the previous Liberal government is passed by the new Liberal-Centre-Conservative coalition. The bill is based on a one-man investigation of whether recombinant-DNA research can be carried out under Sweden's present laws. It proposed that a new body, to be called the Biotechnical Committee, be set up under the present Labour Protection Board, to advise the Board whether specific experiments may be done and under what conditions. It would also follow international events in the field and keep the Board, government and public informed of developments.

According to the Bill, the committee should be composed of specialists in genetics, microbiology, biochemistry and plant breeding; representatives of the Social Welfare Board, Nature Protection Agency, relevant research councils, trades unions and employers' federations. The chairman of the committee should be the Director of the Labour Protection Board. The bill does not specify exactly how many members the Committee should have; but the report on which it is based suggested a composition which put the scientists in the minority. Until a decision on the new system can be taken, there is a moratorium on new recombinant-DNA work.

Predictably, many members of the present Hybrid-DNA Committee (which has eleven members: seven scientists, one research engineer, one laboratory assistant and two Members of Parliament) are very critical of the proposed system. Professor Peter Reichard, the Chairman of the present committee, told *Nature* cautiously that "the new system won't be better than the present one." Other scientists were more forthright. "The new system would be enormously dangerous", declared Professor Lennart Philipson of Uppsala University. "It would be very bureaucratic: totally impossible. How much should you let public opinion aroused by newspapers influence the work of governmental agencies? Everything will depend on the people on the committee. How much common sense will they have?"

Wendy Barnaby

Avowed Nazis attack Brazilian physicists

A group calling itself the Movement for Nazi Renovation (MRN) has physically attacked scientists and intellectuals in Brazil. In a letter sent to the press they "take the responsibility for the terrorist attack upon the house of Mario Schemberg and his wife Lourdes Cedran". Schemberg is President of the Brazilian Physical Society; his wife was beaten up last month at her home by two men. At the same time several other jewish physicists and artists received anonymous telephone calls.

The MRN criticised these people, some "for having international prestige, for being jewish and as such diabolic", and others "for being living filth and all against the nuclear agreement" (between Brazil and Germany). The MRN also declared itself "against the feminist movement which, although pure nonsense, stimulates women to disobey men and have ideas of their own." The letter proclaims that MRN is "in favor of the nuclear agreement and of the arian race reassuming its historical role, specially in the military domain."

"The situation is quite serious", Professor Jacques Danon of the Brazilian Centre for Physics Research in Rio declared in a manifesto signed by all members of the centre: "A professor of sociology from Campinas was abducted last week and forced to drink castor oil . . . What is most worrysome is that the government is trying to minimize these terrorist attacks."

The head of the Security Police of Sao Paulo declared that one should not be concerned about "a joke done in bad taste", while the influential ex-minister of education Passarinho declared that the whole "affair" was unimportant.

The scientific community is apprehensive. The end of formal political repression had indeed led to open criticism of Brazil's gigantic nuclear agreement with West Germany which involves the construction of eight 1000 MW nuclear power stations in the next five years (*Nature* 2 August 79). The physicists presently under attack have all been particularly vehement in their opposition to the government's nuclear option for Brazil.

Recently Professor Cerqueira Leite said that the president of Nuclearbras, the government company which administers the nuclear contract, had gone from being a "nuclearcrat" to being a "nuclearpath" in accusing the physicists of participating in an international conspiracy against Brazilian interests allegedly involving both the United States and the Soviet Union.

The scientists are demanding in their manifesto that the authorities take the situation seriously. In the words of professor Danon, "even if MRN consists of a few crazy individuals, the government must prove that it has the will to act against them; otherwise some of our colleagues will be found dead tomorrow."

Maurice Bazin

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news in brief

Research funds sought for Three Mile Island clean-up: The owners of Three Mile Island nuclear plant said last week that they were negoatiating with the Department of Energy over Possible federal support for the Clean-up following last March's accident as a research project, on the grounds that the work will be "a covenient way of learning more about reactor safety". Mr Herman Dieckamp, President of the General Public Utilities Corportation, told a Senate Committee that the DOE was planning to fund research that could be conducted either at the TMI plant; or on materials from the site for which the government would pay the utility company access fees and materials costs. Typical research projects might include the development of decontamination methods, studies of hot core behaviour and removal and experimental work on damaged fuel pellets. "This is an important opportunity to add to the nation's nuclear expertise", Mr Dieckamp said.



"Remember to keep the computer informed every time you wring out your mop!"

AAAS executive foresees greater state direction over science: "I suspect strongly that the 1980s will see the main directions of science and technology decided by government" Mr William Carey, Executive Officer of the American Association for the Advancement of Science, said in Washington last week. Addressing the annual conference of the Society for Social Study of Science, Mr Carey said that the US was already far down that road, since the federal government paid more than half of the national bill for research and development, and influenced the rest through regulatory, tax and fiscal policy. "The illusion of partnership between government and academic science remains a lovely but abstract image, but it is not (if it ever was) an equal partnership". Domestic and international problems meant that this relationship would be "tortured progressively in the coming years". There was likely to be a rapidly growing social preference for centralised government, at the expense of pluralism and checks and balances. And one of the great tests would be that of holding on to such negotiating authority as science still possessed in its bargain with society.

Birmigham University not guilty in smallpox case: Birmigham University was found not guilty last week of health and safety violations arising out of the smallpox outbreak last year that killed Mrs Janet Parker, a university photographer. Parker was working on the floor of the pathogen laboratory of Professor Henry Bedson and the prosecution alleged that Parker caught the disease from airborn particles carried though a faulty ventilation system. Witnesses for the university rejected the argument saying that they were unable to assign a cause to Parker's death other than "personal contact". The UK Health and Safety Executive who brought the suit called the result "disappointing". The HSE is considering an appeal.

UK medical researchers lobby parliament: British politicians who met with representatives from the Association of Researchers in Medical Science on 8 November said that while they sympathised with the problems of short term contract workers they "were not sure that it was a matter for government". One hundred representatives of research workers in the UK marched to the House of Commons after presenting a petition with 1300 signatures to Mr Mark Carlisle, the Minister for Education and Science. A succession of MPs were "amazed" that they had "never heard of this before" but they wanted explanations about the particular situation of researchers compared to other groups in difficulty, why the trade unions weren't involved and they told the representatives that "researchers need to be very much clearer about they want to do". One MP offered to meet with ARMS and the Medical Research Council but when questioned about the efficacy of such a move said that he "wouldn't want to get into a confrontation with the MRC". As a result of the meeting ARMS will prepare a detailed brief of its proposals for a career structure for non-medically qualified researchers by Christmas.

Two thousand protest reorganisation of French biomedical research: In an open letter to the Prime Minister, 2000 French biomedical researchers have opposed "with profond emotion" the new governmental directives on scientific employment — particularly in INSERM, the national institute of health and medical research. Three hundred researchers demonstrated outside INSERM on 6 November for the meeting of its Administrative Council. According to *Le Monde* the signatories include 195 university professors and hospital doctors, 205 directors of research (including 89 INSERM unit directors), 81 heads of hospital services, 13 presidents of teaching and research establishments, 4 professors of the College de France and "one Nobel laureate". The principle points of contention in the new directives are the age limit on recruitment (27 years for researchers, 30 for doctors), a "forced mobilty" plan which would give the government the power to assign researchers to any laboratory and new restrictions on promotion when reseachers are needed on "priority projects".

Space Shuttle faces further delays — and legal probe: Nothing seems to be going right for the Space Shuttle, which the US National Aeronautics and Space Administration plans to have flying by the middle of next year. Last week another engine was severely damaged during testing at NASA's National Space Technology Laboratories in Bay St Louis, Missouri. No official statement of the impact on the Shuttle programme has been made, but it could delay the first launch, already a year behind schedule, by a further two to three months.

On top of this, the US Department of Justice announced last week that it was investigating charges that Rockwell International, the main contractor for the Space Shuttle, had hidden cost overruns on development aspects of the programme in later production budgets — and that this may have contributed to the delay in the cost-overruns now estimated at 20% above the initial estimate of $5.15 billion.

US Congress approves $20,000 million programme to develop synthetic fuels: President Carter won a major victory for his energy proposals last week when the US Senate approved administration-backed legislation to spend $20,000 million on the development of synthetic fuels over the next five years. The Senate also approved expenditure of a further $14,000 million for conservation measures and the use of solar energy and gasohol — including a commitment to speed the development of fuels from agricultural and forest products, a move enthusiastically supported by the US farming community. The Bill approved by the Senate, which has been sent to the White House for signature following the passage of a similar measure in the House of Representatives last Friday, sets a target goal of 1.5 million barrels of oil equivalent of synthetic fuels a day by 1995. President Carter had initially proposed spending $88,000 million on synthetic fuel development over the five years, but subsequently gave his support to the reduced programme. Included in the programme is an Energy Security Corporation which could build three government owned synthetic fuel plants.

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Views of China are as diverse as its visitors. Here Tilman Spengler, who is responsible for scientific exchanges between China and West Germany's Max Planck Institutes, argues that the positive aspects of the Cultural Revolution have been forgotten, while Ken Hsu, (opposite), an expatriate geologist just back from China, lambasts that period and welcomes its passing. In a series beginning later this year, our China correspondent T B Tang — who has also recently visited China — will describe how science remains an intensely politicised activity, despite the impression in the West that the 'Four Modernisations' are loosening overtly political constraints.

China: the legacy of the Cultural Revolution



Involving workers in science during the Cultural Revolution

"THE Gang of Four", a Chinese solid state physicist told me happily in early 1977, "have always insisted that Marxism provides the foundations for solid state physics. That has changed. Today we can again point out that Maxwell equations are of some importance too".

The scientist had the laugh on his side. The other side, the 'Gang', eponym in today's China for every irresponsible ideological nincompoop, had lost their final battle. With the purge of 'The Four' in October 1976 the Cultural Revolution had for most practical purposes come to an end. As time went by, more and more tales of horror about persecutions and harassments of scientists, and accounts of vandalism in laboratories and libraries were revealed in the Chinese press (and to visiting colleagues from abroad). The message — at least as far as science was concerned — left little room for doubt: the Cultural Revolution hadn't just led to a mess, it had ended up in a (sometimes bloody) disaster.

The stories are indeed frightening even if some of them do contain exaggerations — and they should make some Western observers (including an uncomfortably high percentage of natural scientists) think at least twice how they could have eagerly adopted and retained the image of a happy Chinese scientist, cheerfully abandoning his or her research in, say, astrophysics, and discarding all social privileges to become part of the labouring many.

There may have been some exceptions, like a biochemist I met last year in Shanghai who preferred working on pesticides in the countryside to solitary confinement in his unit (he is by training an expert in neurophysiology). And there are others who admit that "it did us good" to experience the hardship of having to plant rice sprouts ten hours a day in a paddy field, the common lot of many of those who provide the funds for whatever research is being conducted in the country's laboratories. On the whole, however, the leaders of the Cultural Revolution were apparently singularly unsuccessful in their dealings with China's scientific community. The revolutionaries had a clear idea of what they did *not* want: elitism among intellectuals, international orientations, discipline rather than project oriented research, a system of social values that would perpetuate the division between manual and mental labour, material rewards, and so on. And they hoped to overcome all the traditional obstacles to a "planned science" geared to the country's socio-economic developments by creating the model of a selfless scientist who, by the very nature of his motivation 'for the cause', could do without the common academic incentives (reputation and to lesser degree remuneration).

As we have seen, sticks rather than carrots were used — sticks that Red Guards and a new generation of cadres had little hesitation in applying to individuals and institutions alike. And the Academy of Sciences, hitherto the most powerful and prestigious research organization of the People's Republic made no exception to the rule.

But was that all there was to it? Is the Chinese press justified in blacking out the decade between 1966 and 1976 as the uncontrollable reign of a few malevolent poltergeists, beset by chauvinism (or at least parochialism) and bent on keeping China in a state of unenlightened autocracy?

A number of facts contradict this new projection. Take, for example, the issue of internationalism. Most of the contracts between the Chinese Academy of Sciences and foreign research organisations, leading to exchange programmes of varying intensity, were begun long before October 1976. Another example is the issue of fundamental research, claimed to be one of the main targets of the science policy of the 'Gang'. Yet high-energy physics has been a priority in Chinese science politics ever since 1972 — and it takes a rather sophisticated understanding of the 'needs of the masses' to include high-energy physics. There were also a number of institutes within the Academy that worked very well, including the Institute of Botany, located in Kunming and set up to investigate the tropical plants of Yunan province. Even by western standards, this institute is (and was) well equipped, combining extensive empirical researches with highly competent analyses in pharmacology. Others were the Institutes for Vertebrate and Invertebrate Palaeontology in Beijing (Peking) and Nanking, or, to move to the social sciences, the Academy's (now the Academy for Social Science's) Institute for Archaeology, again in Beijing. Of course, these examples cannot be multiplied at will,

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but they might make us pause for a moment before we join the chorus of lamentation.

And one should not pass lightly over the various mass campaigns to integrate research, education, health and safety, and production. Again a few examples: in mathematics, it was the "Movement for Operational Research" (yunchouxue yundong), and in oncology the emphasis on epidemiology. One could further add mass experiments and observations in plant pathology and earthquake research.

Let me briefly elaborate the last-mentioned example. Even before the Cultural Revolution, earthquake research had included a variety of specialists, such as mathematicians, geophysicists, geologists, seismologists, architects and historians. Their projects ranged from the adaption of seismographic scales to the pecularities of the structural engineering aspects of Chinese buildings, to the compilation of a chronology of earthquakes in the history of China which would then be used to divide China into different earthquake regions, as well as planning apartment houses and public buildings which would permit maximum resistance to earthquakes. During the Cultural Revolution this project was extended to include research teams of botanists, zoologists, and meteorologists. They investigated striking changes in fauna or flora which had traditionally preceded earthquakes, and changes in the ozone content of the air, which likewise serves as an empirical indicator for the threat of an earthquake.

Taken by itself, the addition of the last named group would hardly have constituted any startling departure from the previous forms of organisation. The decisive difference resulted from the widening of the area of inquiry to a level where literally "the masses" could participate. This type of horizontally and vertically integrated research was aimed not only at coordinating many individual talents to deal with a given problem (a social problem translated into a scientific problem), but was also intended — and this was the special message during the Cultural Revolution — to emphatically stress the importance of practical experience, its identification with science and its value for new orientations of action. Thus it encouraged a significant number of peasants to set up and maintain, in their own villages or production brigades, observation stations for early warning signs or earthquakes, on which they provided data. This brought about quite a considerable change of consciousness. Ordinary day-to-day routines acquired the dignity of scientific observations, and as a result reduced much of the traditional mistrust in, and resistance to, 'science' as something alien to their concerns. Equally important, the adherence to mythological or cosmological explanations which had enjoyed widespread popularity in the Chinese countryside, declined decisively. Natural phenomena had become interpretable in a new context. And this had a third result: the increased survival chances of a people who had learned to replace fatalism by practical planning.

The case of earthquake research probably serves best to illustrate the promises and the limitations of the Cultural Revolution's policy. The promises I have demonstrated: a research that would overcome disciplinary boundaries, answer to a huge social demand, integrate the participants and demanded no great financial investments.

But the limitations are equally clear. Taken as a paradigm for the only legitimate type of research, it would yield results only in those problem areas where everyday data play an mportant part in the solution, thereby leaving most of the 'mature' disciplines at the wayside. And 'maturity' is under no political conditions an argument for disposal, as the Chinese have had reason to find out.

Tilman Spengler

Tilman Spengler is a sinologist working at the Max Plank Institut fur Erforschung der Lebensbedingungen der Wissenschaftlich — Technischen Welt, Starnberg, West Germany.

THE vast arms of China's State Bureau of Geology (SBG) embrace 29 provincial bureaus, more than 20 research institutes, numerous exploration teams, and several institutions of higher education in earth sciences in addition to its headquarters at Peking. Earlier this year, I spent five and a half months as a 'guest expert' of the SBG, and I visited several of these branches in my capacity as a non-paid consultant to help 'modernise' geological activities in China. Usually oral presentations by Chinese colleagues, on which I was expected to give my opinions freely, were followed by outdoor discussions in the field. I would also sit with senior scientific staff and administrators to work out plans to orient future activities. Other duties involved lecturing, including a four-week course on plate tectonics and sedimentology to more than 400 geologists from 91 organisations and hours of subsequent informal discussions.

It has been estimated that China has more than 20,000 geologists. I could not have met more than 10% and talked to more than 1% of the total, and my sampling was not random, so my evaluations should be biased on the optimistic side. However, the reports I heard were not show-pieces, as were presented to us in 1977 when I was a



member of the visiting delegation of the International Union of Geological Sciences. I am, therefore, inclined to believe that I did manage a glimpse beneath the superficial during this particular trip.

If I had to use one word to characterise the level of the geological profession in China, the word would be uneven. Our Chinese colleagues are rather up-to-date in offshore exploration geophysics, exploiting much modern equipment, and it is not surprising that their efforts here have been repeatedly successful. On the other hand, sedimentologists in the same office were still using the methodology of the 1930s. *Erratic* is an apt word to describe the equipping of laboratories. They are commonly well endowed with instruments to analyse material properties of samples, but inadequately equipped to gather data that promote understanding of principles, or of geological processes. In an organisation where Quaternary studies form the essential basis for stratigraphical interpretation of potentially oil-bearing strata, its laboratory is not equipped for paleomagnetic studies and does not have a scanning electron microscope for

Ken Hsü — pictured above right — is a Chinese Scientist working at the Eidgenössische Technische Hochschule, Zurich. He visited China last August.

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nannofossil investigations. Its pollen-laboratory is also inadequate, yet a neutron-activation apparatus has been acquired for microchemical analysis.

There is also a woeful lack of coordination among institutes. Each organisation would like to have its own 'small and complete' laboratories. Administrators were often faced with requests from several institutions for the same expensive equipment, which none of them could utilise fully. The idea of joint ownership and joint research projects among various SBG units was not explored, not to mention cooperation between SBG geologists and those of other systems. The situation became painfully obvious at Tsingtao, where I had contacts with marine geologists and oceanographers from four different systems. Funded separately, each may be able to purchase equipment of the 1950s and 1960s vintage, yet none considered seriously the possibility of combining resources to set up a common up-to-date facility for a national programme on oceanography.

Less visible, but more serious than the problem of modernising equipment is the need for modern methodology. For example, the basic tools of a field geologist — hammer and compass — have remained unchanged for the last century, and it was thus difficult to convey the idea that the methodology for field geology had made advances until we went to the field together. There, I could point out the irrelevance of worrying if a boundary between formations X and Y should be drawn at the base of a bed A, or a few metres higher. Nevertheless, it was not an easy task to persuade our young colleagues to give up their naïve reliance on specific indices, for example that glauconite proves marine deposition even if only a trace is found after a week of searching.

Some Chinese regard this tendency to equate scientific research with routine data-collection as a relic of the Soviet influence. Following the Soviet lead, the People's Republic has adopted the policy of separating teaching (universities), research (Academia Sinica), and practice (Ministry of Geology). There is also a tendency towards premature specialisation: a student has to choose a major in one of the 10 or 15 subdepartments (such as hydrogeology or geophysical prospecting) upon entering the university.

The Soviet ideology had also a depressing effect on senior scientists who had studied in Europe or North America. Many in fact retained their positions after the liberation, but they did not seem to have received the complete trust of Chairman Mao, who repeatedly referred to the need to re-educate the bourgeois intellectuals. Some were down-graded in the aftermath of the 'Let a hundred flowers blossom' campaign of 1957; others were weeded out during the anti-rightist campaign of the late 1950s and early 1960s. Virtually none of the senior scientists survived the Great Proletarian Cultural Revolution (GPCR) of 1966-1976 unscathed, but the government was so successful in their cover-up that I learned very little of the actual happenings of the GPCR when I visited China in 1976 and 1977. The present governmental policy is not to hide the truth, however, and many of the former victims can now come out with all their woes. Physical punishment, mental torture, forced labour, and imprisonment were the lot of all intellectuals who were occupying high academic positions at the start of the GPCR; I encountered no exception among my relatives, friends, and acquaintances. Many died of suicide, or of poor health.

I consider that the persecution of the intellectuals by the Gang of Four during the GPCR was comparable to the persecution of the Jews by the Nazis during the 1930s: Red Guards or rebel cliques went about freely to search homes, to beat, insult, or imprison people. They set up kangaroo courts which could pass death sentences, without any regard to due processes of the law. There were, for example, ten such 'courts' in the University of Nanking alone! Those, such as my sister, who sat in isolation cells could see no one, talk to no one, and read nothing but the four volumes of Mao's Selected Writings. One of my former schoolmates at the University of California, who returned to serve China in 1954, was kept in a damp cellar for six and a half years, because the rebel clique believed that only a CIA spy could leave the comfort of the US to return to China: patriotism had no meaning for those cynical opportunists. (This friend is now nursing a weak heart complicated by rheumatism.)

Also persecuted during the GPCR were the middle- and high-level cadres. According to the slogans then, these cadres were the "revisionistic capitalist-roader" who enabled the "reactionary academic authorities" to betray the Revolution. Very clearly, Lin-Piao, the Gang of Four, and their followers got rid of most of their opposition. Not only were all the province chiefs changed, but all administrators down to the level of administrative assistant of a research institute or university department were at one time or another imprisoned or sent to labour camp. Productive work was suspended completely for the three years to 1969.

When operations were resumed, the men in charge were the so-called 'Worker's Representative', or 'Soldier's Representative', who had no knowledge of the work to be done. The dictatorial Soldier's Representative of the SBG, for example, declared that the practice of doing field-work during the summer and laboratory-work during the winter was a bourgeois soft-practice. Therefore, he forbade the return of field geologists to the laboratory even when the ground was snow covered. To carry out Lin-Piao's order for decentralisation, research institutes and universities were sent to the country in the provinces, where there were not enough buildings or facilities to permit normal operations. The famous Geological Institute of the University of Peking, for example, was moved to a village in Hupeh, where there was not enough space to open the packing cases, not to mention the inadequate classrooms and housing for staff and students. Consequently the institute had to remain inactive until it was sent to Wuhan after the downfall of the Gang of Four.

The lot of the young scientists was not much better. They were obliged to join one or another of the rebel cliques, which were often engaged in armed battles. At the height of GPCR, young and old professionals were forced to perform the 'Loyalty Dance' and to sing songs like schoolchildren in praise of the Great Chairman. For several years, they could not read anything other than the four volumes of the Selected Writings. They could gradually return to their work in the early 1970s, under the dictatorship of the solider's representatives who thought of geological exploration as battles to be fought, and issued orders such as: "Take that hill yonder!" The Chinese scientists were thus completely isolated from the international community for ten years.

I may have painted a horror picture of the past, and made an unflattering diagnosis of the present. However, I am optimistic of the future. The present regime has indicated the will to search for solutions to problems, and in this second campaign of "a hundred flowers", there is a liberation from fear. Free and creative thinking is now tolerated, even encouraged, rather than condemned as 'capitalistic decadence'.

During my discussions with administrators, I was encouraged by the fact that funds are available, and steps and being taken to modernise two sedimentological laboratories of the SBG. New centres with the most modern equipment will be established for paleomagnetic and for stable-isotope studies. With the concentration of financial investment and manpower of a socialist system, projects which are not practicable in capitalist countries could be carried out in China. For example, deep drilling of lakes is to take place in order to obtain continuous record of climatic changes during the Quaternary; and continuous coring of a four-thousand metre deep offshore well is scheduled to provide a Neogene reference section — something no capitalistic oil company could be persuaded to do.

My greatest consolation, however, is an awareness of the intellectual quality of the geologists and the administrators to whom I talked. After having had to fight tradition-bound arrogance for the last dozen years here in Switzerland, it is refreshing to find people willing to listen to logical agruments for innovations.

Ken Hsü

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"We have a greater degree of social responsibility than UK scientists"

Joe Schwartz talks to three Chinese scientists visiting the UK about their experiences of research and politics in Britain and China.

MS KAO TSE, aged 46, a chemist from Shanghai, Mr Hsu Tan-lin, aged 38, an electrical engineer from Peking and Mr Tsang Shao-hsien, aged 40, a geophysicist also from Peking are three of a group of 12 scholars in the age group 35-50 that are now visiting the UK. Sipping green tea poured out of a large thermos flask, we discussed differences between Chinese and UK science in the plain quarters of the Chinese cultural mission in West London, far from the smart foreign embassies of South Kensington.

The training of all three has been on the European model. Ms Kao, the daughter of a university professor, attended Futan University, Shanghai's largest with 4500 students. She specialised in chemistry, the curriculum consisting of inorganic chemistry, physical chemistry and organic chemistry with mathematics up to calculus, two years of physics and a "small amount" of quantum mechanics — a standard Western course of study in 1953 the year she graduated. After teaching in Futan University for a year she attended Peking University for two years post graduate training in inorganic chemistry. She rejoined the staff at Futan University with an MSc in 1956 as an assistant instructor and was promoted to lecturer in 1959, a position she has held since.

Kao has worked on a variety of research projects but since 1973 her main work has been on the use of rare earth zeolites as catalysts and as molecular sieves. Zeolites are natually occurring alumina silicates found particularly on the ocean floor that are used as catalysts in cracking crude oil an area of international concern. Zeolites are also useful as molecular sieves in petroleum by-product refining. Kao is spending two years at Imperial College in London in the laboratory of Dr Lovat Rees, a UK specialist in zeolite research. "I came from a large research group of 25 people in Futan University. We have similar facilities but on a smaller and less well developed scale. At present China must import its larger instruments so we are behind particularly in the use of Mossbauer spectroscopy and in the use of computers.

"I didn't feel at all strange working at Imperial College and I was able to start work right from the first day. We still have a lot to learn from scientists in the UK — more advanced experimental methods and a broader knowledge of related fields. But there isn't that big a gap."

Hsu, the son of a Shanghai metal worker is working on aircraft navigation technology at Imperial College. His home institution, the Peking Aeronautical Insitute sent him to the UK "to get current with the most advanced technology in the field." His brief is digital signal processing using large integrated circuits and computer processing. Digital signals are considered to be far more reliable and easier to work with than analogue signals. Hsu thinks that China can catch up in this field within five years.

He is conversant with the debates about indigenous versus imported technological development. "The government's policy is still one of self-reliance because you can't have political independence without economic independence. We want to introduce microprocessors without becoming dependent on foreign imports. There are already many people in China working on microchip technology and we now can produce some of the chips. We are using the technology that we buy as learning equipment with the ultimate aim of producing it ourselves."

Tsang Shao-hsien's is an earthquake specilaist. His work as lecturer in geophysics at Peking University has concentrated on earthquake prediction. It is an area with extensive and unique mass participation. According to Tsang there are now 10,000 professionals and "many more" amateurs engaged in this research with every province having its seismology bureau and brigade. In this field Tsang feels that the postive aspects of the Cultural Revolution were felt more strongly than in more laboratory based work. "Earthquake prediction is in a primitive state all over the globe. In China, the professionals understood that they needed help from the people. The Cultural Revolution posed the question of theoretical work versus parctical work and before 1975 'mass work' instead of professional work was emphasised. In 1973 our efforts were to build more instruments and organise more people. China has good social organisation and earthquake research lends itself to this since the science and the socio-political work can be organised together."



Workers at the Shanghai Machine Tools Plant: a model for training technicians

Tsang thinks, however, that theoretical work has been neglected. He has been in the UK for two years familiarising himself with UK theoretical work in the field.

On the wider questions of science policy, education and economic development Kao, Hsu, and Tsang were forthright but at the same time circumspect in the expression of their views. All three believe in quality education. Kao was particularly impressed by recent UK Conservative policy in this area. "I think the Thatcher policy of emphasising aid to the independent schools is important. Some schools should be better than others. In China we have a policy of selecting 'key schools' to make an example for the others."

None of the three saw any danger in the possible domination of education by a small group of intellectuals and party officials. Present policy is that graduates from middle school at age 16 can apply for three years further schooling through competitive examinations set by each city. This is to be compared to policies adopted during the Cultural Revolution when university places went to students elected from their place of work. The example of the Shanghai Machine Tools Factory was important enough for Mao to refer to it in a policy statement on science education in 1968: "It is still necessary to have universities; I refer here mainly to colleges of sciences and engineering. However it is essential to shorten the length of schooling and to make it more political as the Shanghai Machine Tools Plant has done in training technicians from among the workers. Students should be selected from workers and peasants with practical experience and they should return to production after a few years study".

Kao, Hsu and Tsang were familiar with this policy but dismissed it as being "unrealistic." "We would like to enroll all middle school graduates but we must select" says Tsang. Hsu feels that the arguments put forward during the Cultural Revolution were "worthless" and that scientists must "concentrate their efforts on doing scientific work" instead of "being occupied with politics."

When asked whether they saw any differences in the way the UK organised its scientific work compared to China, Kao answered for the group. "In China we tend to work more collectively. It is unheard of for an individual to work alone whereas this is quite common in the UK scientists. Here in the UK, scientists do their work because it interests them. But in China we have adopted the saying "serve the people" as our guiding policy. We believe that the work should be useful first and interesting second. Otherwise it is not good for the human being." □

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One culture bridging two

Samuel Ting, discoverer of the J particle, is a Chinese scientist living and working in the West. Here he talks to **Christine King**

SAMUEL Ting is an American citizen by "an accident of birth". His parents, both professors, were on a visit to Ann Arbor, Michigan, from China in 1936 when he was born. Ting says "my parents had hoped that I would be born in China, but I was born prematurely. Two months after my birth we returned to China." During those early years, China was a country torn by war, so formal education was impossible until he was twelve. Ting remembers now that this lack of continuity in education was compensated by meeting the many scholars who frequently visited their home. Ting's present reputation for hard work and being a highly demanding task-master has its origins perhaps in these early years.

"Since both my parents were working, I was brought up by my maternal grandmother, who was a very courageous and determined person. My grandfather had lost his life in the first Chinese Revolution. After that my grandmother returned to school, became a teacher and brought up my mother alone. When I was very young, I often heard stories from them recalling the difficult lives they had, and the great efforts they made to provide my mother with a good education. Both of them left an indelible impression on me."

Ting attended schools in China and later Taiwan, and decided to return to the US to continue his education at the University of Michigan. "At the time, I knew very little English and had no idea of the cost of living in the US. In China, I had read that many American students go through college on their own resources. I informed my parents that I would do likewise. I arrived at Detroit Airport on 6 September 1956 with $100, which at the time seemed more than adequate. I was somewhat frightened, did not know anyone and communication was difficult". These were hard years for Samuel Ting, competing constantly for scholarships.

In 1963, with a doctorate and as a Ford Fellow, he went to CERN (the European organisation for nuclear research in Geneva) and came under the guidance of Guiseppe Cocconi, working with the Proton Synchrotron.

After this initiation into European high energy physics, Ting returned to lecture at Columbia University. Here he worked with leading physicists including Steinberger, Schwartz, C.S. Wu, and T.D. Lee. It was during these years at Columbia that his — then unfashionable — interest in electron-positron pair production began.

At the Cambridge Electron Accelerator an experiment on e^+e^- pair production in photon collisions with nuclear targets appeared to show a violation of quantum electrodynamics. (QED is the relativistic quantum theory of electromagnetism; it remains the most accurately tested of all physical theories.) Ting studied the apparent violation with increasing interest and eventually asked Willi Jentschke of the DESY (Deutches Electronen Synchrotron) laboratory in Hamburg about doing a pair production experiment at Hamburg. By this time Ting was fully committed to investigating the decay of photon-like particles, and searching for new particles which decayed to electron or muon pairs. In 1971 he returned with his group to the US and began experiments at the Brookhaven National Laboratory. It was here, in late 1974, that Ting and his group found evidence of a new, totally unpredicted and long-lived heavy particle, the J or psi-particle, which was subsequently shown to contain charmed quarks. For this work, Ting shared the Nobel Prize for physics in 1976 with Burton Richter.

With Ting's growing reputation as a leading physicist have come new problems. In the present political climate of growing intercourse between the US and China, Ting's participation is inevitable. His group at DESY was amongst the first to receive physicists from the Institute of High Energy Physics in Peking to work in the West. The Chinese Press consistently refer to visiting scientists like Ting, and fellow Nobelists Yang and Lee, as Chinese-American or just American. Ting however, is a totally apolitical figure. He categorically repeats "I am not interested in politics and never have been; I have never voted (in the US).

On his trip to China a few weeks ago, Ting was feted by the Chinese Academy of Sciences (CAS), and as on previous visits, talked privately with Chinese leaders Chairman Hua and Vice-premier Fang Yi, who is also president of the CAS. They talked of China's modernisation programme but, Ting says, they have never discussed political issues "probably because they already know my views".

Ting believes that since he goes to China to communicate with Chinese physicists, there is no time to sightsee. Each of his visits has been widely reported in the Chinese press, and every time his lectures have been attended by thousands. At first he had difficulty lecturing in Chinese (he has spent more of his life on the West than in China): "my audience of several thousand was hardly able to contain their mirth, and corrected me several times on scientific terminology. But I persisted and now it's no longer a problem."

To the outside world, China's decision to pursue high energy physics as part of its modernisation programme, is, to say the least, puzzling. "As I see it" said Ting, "although particle physics appears very complex, its fundamental principles are nevertheless extremely simple . . . Major discoveries are made by only a few individuals and China has plenty of these. It is an expensive pursuit, but it's also the most fundamental — and it's cheap by comparison to what's spent on defence, say." He believes that the Chinese will maintain their independence in high energy physics. But China's future scientific progress is not a subject which he, or anyone else outside China would feel qualified to predict.



Samuel Ting: not interested in politics

Ting belongs to a new generation of jet-setting academics, spending much of his time at DESY, where he is pursuing the search for the intermediate vector boson at PETRA, currently the world's most sophisticated electron-positron colliding beam device. To keep abreast of his commitments at MIT where he is professor of physics, he flies between Boston and Hamburg on average once a fortnight.

Despite his reputation for working his team hard, no-one works harder than Ting himself. How does life in Hamburg differ from his life in the US? "My life in Hamburg is work, work and more work. As for my team, I try to choose only those who also like to work hard." Interviewing Ting can be a formidable experience if one expects any human foibles — in his own words, he does not smoke, drink or gamble, nor does he read fiction. His family now lives in the US, although the children spent many years in Germany. When he arrives home, do his children complain that they don't see enough of him? "Yes, all the time." Like many Chinese fathers, Ting is determined that his children should put their studies first. When I remarked that in talking of his children, he had repeatedly said only how he expected them to work hard and do well at school, Ting gave a rare laugh. "It's true — around the block where I live, I'm known as the 'bad guy', because I'm so strict with them."

When Ting is not pursing theoretical particle physics, his predilection is very much towards history. He regards Faraday as a notable historical figure, admiring not only his great breadth of vision but also the difficult and determined path by which he achieved his mastery of science. To be seen as another Faraday — no doubt that would be Samuel Ting's dream. □

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news and views

The solar wind connection

from Alan Johnstone

THE solar wind is deflected around the Earth by the Earth's magnetic field. The electrical currents which flow in the boundary layer between the solar wind and the magnetosphere shield the solar wind from the Earth's magnetic field. In 1961, Dungey suggested, that, if the magnetic field, of solar origin, in the solar wind was opposite in direction to the Earth's field at the boundary (called the magnetopause) then the two magnetic fields would diffuse into each other and some geomagnetic field lines would become connected to solar wind field lines (Fig. 1). The geomagnetic ends of the field would be dragged behind the Earth by the solar wind to form a long magnetospheric tail. In the tail the geomagnetic field lines would become reconnected again, and return to the interior of the magnetosphere. The plasma flow driven by this process transfers energy from the solar wind to the magnetosphere, and if sufficient magnetic connection occurred it could produce magnetic storms and auroras.

It is now known that some geomagnetic field lines connect to the solar wind, that the pattern of plasma flow is similar to that driven by such a process and that geomagnetic activity is greater when the solar wind is more nearly opposite the Earth's field (that is, southward directed). Until recently there has been no evidence on the microscopic scale from spacecraft that connection takes place at the magnetopause.

The fields and plasma flow near a region of magnetic connection are shown in Fig. 2. The shielding electric current J flows out of the paper. In a closed magnetosphere the magnetic field on both sides is parallel to the surface. The signature of magnetic connection is a small component B_n normal to the boundary. Although spacecraft magnetometers are very accurate the local surface plane of the magnetopause cannot be determined from a single spacecraft, or even a pair like ISEE (International Sun Earth Explorer) 1 and 2, so that a small normal component cannot be reliably identified.

Another indication of magnetic connection is an electric field E tangential to the magnetopause. The electric field is more difficult to measure than the magnetic field in tenuous plasma and the first measurements at the magnetopause were not made until 1977 by ISEE 1, and 2, more than 13 years after the first observations of the magnetic field. Mozer *et al.* (*Geophys. Res. Lett.* **6**, 305; 1979) have reported the existence of a tangential electric field of the order of 2 mV m^{-1} during a series of crossings of the magnetopause in the subsolar region. There were large fluctuations, taking the value to zero on some occasions.

Heikkila (*J. Geophys. Res.* **83**, 1071; 1978; unpublished work, 1979) has argued that a crucial test for magnetic connection is to find what happened to the electromagnetic energy dissipated in the magnetopause, where $E.J$ is positive (in the particular planar geometry of Fig. 2, E is parallel to J). In the case reported by Mozer *et al.* (*op. cit.*) the power dissipated locally is estimated to be 70±20 W km^{-2}. This energy must go into the particle distribution. According to theory, plasma flows from both sides towards the boundary where it is accelerated parallel to the surface by the electric motor force $J \times B_n$. In more picturesque terms, it is catapulted out of the region by the tension in the magnetic field lines. Heikkila (*op. cit.*) showed that the energy deposited in the particle distribution should be observable whether or not this simple picture is correct. Yet, despite many magnetopause crossings by several spacecraft carrying suitable instruments, no part of the particle distribution had been found to be carrying the excess energy until a crossing described in this issue of *Nature* by Paschmann *et al.* (page 243). They have found a crossing where a high speed flow in the magnetopause is observed by both ISEE spacecraft. The flow velocity of plasma in the layer was 450 km s^{-1}; much higher than flow velocities in the adjacent magnetosheath and consistent with the simple picture of acceleration by a magnetic catapult. They estimate a power dissipation of 78±24 W km^{-2}, almost the same as Mozer *et al.* found from electric and magnetic field measurements. Unfortunately, during this crossing, the electric field instrument,

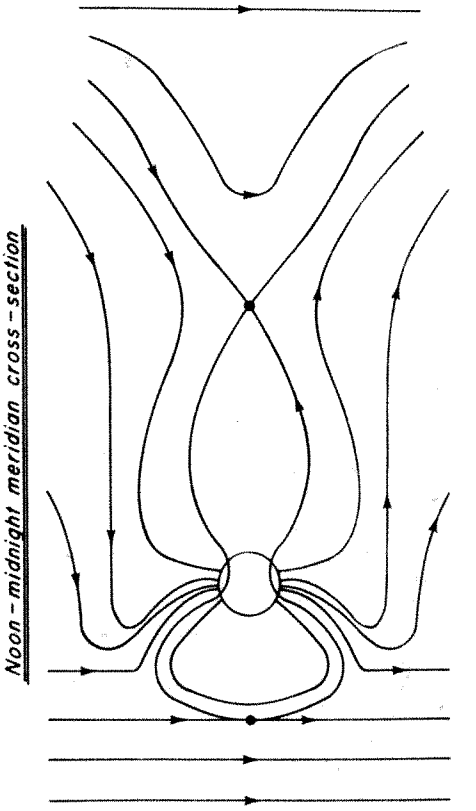


Fig. 1 The magnetic configuration of an open magnetosphere in a southward-pointing, interplanetary magnetic field as proposed by Dungey.

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which was on the same spacecraft, was in an unfavourable mode. During the crossing reported by Mozer *et al.* the same particle detectors show no sign of the acceleration (Heikkila *op. cit.*).

Although the latest result of Paschmann *et al.* provides strong support for magnetic connection it also reinforces the criticisms of Heikkila (*op. cit.*). If the effect is so easily detected in this crossing why has it never been reported before? If magnetic connection occurs it can hardly be such a rare event as the present observational statistics imply. Is it confined to a small region of the magnetopause or are there special conditions yet to be identified? Other examples, which Paschmann *et al.* say have already been found in the ISEE data, may provide the answers and help us find out what controls the rate of energy input from the solar wind into the magnetosphere. □

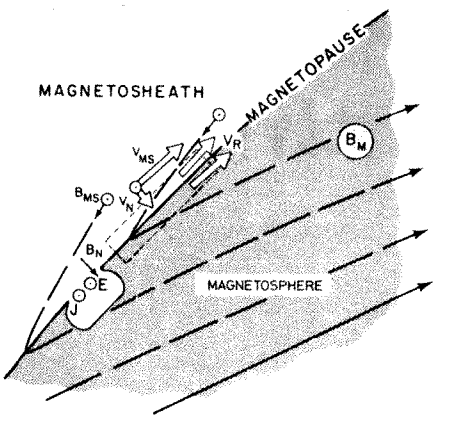


Fig. 2 The configuration of magnetic (B) and electric (E) fields and plasma flow (V) (broad arrows) when magnetic connection occurs at the magnetopause. The enhanced flow caused by acceleration in the layer, V_R, exceeds the normal flow of solar wind plasma past the magnetosphere V_{MS}.

Immunoglobulins and histocompatibility antigens

from Arnold Feinstein

THE family of antibody molecules, or immunoglobulins, for all its magnificent variety and versatility of structure, function and specificity, has evolved by the multiplication of a gene coding for a single ancestral polypeptide sequence, corresponding to a size of about 12,000 molecular weight. As a result of tandem duplication, followed by limited divergence, four or five homologous sequences can be seen repeated along the heavy polypeptide chains of antibody molecules. The κ or λ light chain found in association with each heavy chain is formed from two such homologous regions. In both heavy and light chains hypervariable stretches conferring antigen-binding specificity are confined to the most N-terminal homology region, the so-called variable region. Crystallographic studies of a few immunoglobulins have revealed that variable and constant homology regions are independently folded similarly, but distinguishably, into individual domains.

What makes it quite certain that the chains of all known antibodies are strings of such similarly folded domains is that all the characteristic conserved features of the folded domain, seen by crystallographers, are easily detected in each of the homologous sequences recurring along all the known immunoglobulin polypeptide chains (see Beale & Feinstein *Q. Rev. Biophys.* **9**. 135; 1976). These features include the β strands which form two β sheets, the bends between the strands, and the disulphide bridge lying between the two sheets and linking them. In this way we have the rare good fortune to be able to come close to the tertiary structure of an immunoglobulin domain from a knowledge of only its primary sequence. Now that the sequences of more proteins are becoming available, the basic immunoglobulin domain structure seems to be cropping up in several unrelated membrane proteins.

It has been evident for some time that another remarkable family of molecules, the classical transplantation antigens (the histocompatibility antigens), are in part coded for by genes which have evolved from the same ancestral immunoglobulin gene although the two loci are completely unlinked. These highly polymorphic antigens are present on most cells, and are extremely important, not only as the critical targets in graft rejection, but also for their involvement in the recognition by cytotoxic T lymphocytes of viral and other cell surface antigens (see *News & Views* **274**, 12, 840; 1978). The histocompatibility antigens consist of two non-covalently associated polypeptide chains, the heavier one, which carries the alloantigenic specificities, being a 44,000 molecular weight glycopeptide; the other subunit, β2 microglobulin, has a molecular weight of 12,000, and its invariant amino acid sequence was found, surprisingly, to be homologous to that of a constant immunoglobulin domain, including the characteristic disulphide-bridged loop. Moreover, all the three-dimensional structural features of this domain referred to earlier can be recognised.

In antibody molecules constant domains regularly associate in pairs, so that when two disulphide-bridged loops of appropriate size were seen in the heavy chain of the transplantation antigen, it seemed very likely that at least one of these looped regions would be folded like an immunoglobulin domain, and would be associated with β2 microglobulin. Indeed, the familiar features of a constant immunoglobulin domain could already be seen in a tentative sequence of one of these regions, in a human histocompatibility antigen, which was published last year by Strominger and his colleagues.

In this issue of *Nature* (page 266) Orr *et al.* present the complete sequence of this region, referred to as ac2, and confirm in detail its immunoglobulin-like structure. But what of the other more N-terminal disulphide-looped region? (see Fig. 1 of Orr *et al.* for the relative arrangement of the regions). The sequence of this region too has now been published (*Proc. natn. Acad. Sci. U.S.A.* **76**, 4395; 1979) and it is disappointingly apparent that, apart from the sequence around the most N-terminal cysteine, the overall pattern of conserved features typical of an immunoglobulin domain are not clearly seen. Thus, even if this region were derived originally from an ancestral immunoglobulin gene, it has adaptively lost a number of its features. For this reason, ac2 is a more likely candidate for the region with which β2 microglobulin is associated.

There is however, certain to be a concerted effort to predict a plausible structure for the region N-terminal to ac2, following the realisation that this part of the molecule is important in T cell recognition. This awareness has been highlighted by studies of mutant mice, where dramatic functional changes have been correlated with single amino-acid substitutions in this region (see Kohn *et al. Immunogenetics* **7**, 279; 1978). Not only can such mutations lead to graft rejection, but the specificity for T cell recognition of viral antigens seen in association with the original and with the mutant molecule can be quite different.

It appears that each of the different types of polypeptide chain known to contain immunoglobulin or related sequences is controlled by one of five genetic regions, which are not detectably linked in the species studied. It seems that we can now add a sixth. Thy-1 is a glycoprotein which has been isolated by an Oxford group from rat brain, but it is also abundant on rodent thymus cells. Its sequence indicates that Thy-1 resembles a single immunoglobulin domain (see Campbell *et al.* this issue of *Nature*, page 341). Although the function of this molecule is unknown, we should in general resist the expectation that invariant proteins structurally related to immunoglobulins will have specific recognition properties analogous to those of the specialised variable domains of antibodies. We do not know what function the ancestral polypeptide served, but we have every indication that it owes its extensive proliferation, unique in mammals, to its striking adaptability to a variety of functions.

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Georg Wittig — virtuoso of chemical synthesis

The 1979 Nobel Prize for Chemistry was awarded jointly to Georg Wittig and Herbert C. Brown. Here Robert Shaw describes Georg Wittig's achievements in synthetic and mechanistic chemistry.

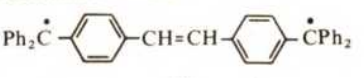
GEORG Wittig was born on 16 June, 1897 in Berlin and after his *Abitur* hesitated between chemistry or music; luckily for chemistry he chose the former. His love of music has remained with him however, and in his earlier days he was an accomplished pianist. I should add that he has also been a keen mountaineer with many difficult ascents to his credit.

He commenced his study of chemistry at the University of Tübingen in 1916 but war service almost immediately intervened. He returned (on release from a prisoner of war camp in the UK) to the study of his chosen subject working under Karl von Auwers. In 1926 he obtained his *Habilitation* at the same university. From 1923-32 he was Teaching Assistant (first under von Auwers, then under Meerwein) at the Chemical Institute of the University of Marburg. In 1932, he went to the Technical High School in Braunschweig as *ausserplanmässiger* Professor. He stayed there until 1937, when he became *Extraordinarius* in Freiburg. In 1944 he became *Ordinarius* at the University of Tübingen and left there in 1956 to become Director of the Chemical Institute of the University of Heidelberg. In 1965 he became Professor Emeritus but continued to co-direct the Institute for Organic Chemistry in Heidelberg for a further two years. He has remained active since. This is a bare outline of a brilliant scientific career spanning half a century.

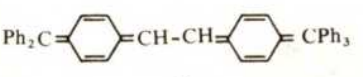
Georg Wittig has received many honours and it is appropriate that his long and distinguished career has been crowned by the award of the Nobel Prize — a recognition which was long overdue.

If the impact a scientist makes on his peers is measured by the achievements which carry his name Wittig has scored a hat-trick: the Wittig hydrocarbon, the Wittig rearrangement and the Wittig reaction — an achievement that has been matched by few.

Wittig's scientific work began with studies on γ-pyrones. Investigations on free radicals then followed, in particular, of polyphenylated hydrocarbons. In an attempt to prepare a biradical (1), the Wittig hydrocarbon, bis[4-(diphenylmethylene)cyclohexadienyliden]ethane (2) was obtained in 1936.



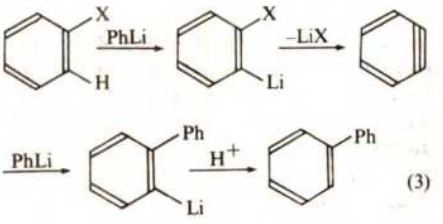
(1)



(2)

For these studies Wittig used phenyllithium, first used by him in 1931; this reagent recurs again and again in many of his most important discoveries. Wittig called it his *Wünschelrute* (divining rod). His work with organolithium compounds and related organometallic reagents (following earlier work by Schlenk and Bergmann) establishes him, together with Henry Gilman and Karl Ziegler, as one of the fathers of organometallic chemistry of the main group elements.

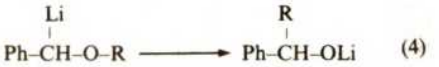
Phenyl-lithium is a very strong base and nucleophile. Both of these properties are of importance in its reaction with halogenobenzenes, PhX (X = F, Cl, Br) to give diphenyl, PhPh. The observation that fluorobenzene (X = F) was the most reactive in this series led to the following reaction scheme, including the postulate in 1942 of the then revolutionary idea of a dehydrobenzene (benzyne).



(3)

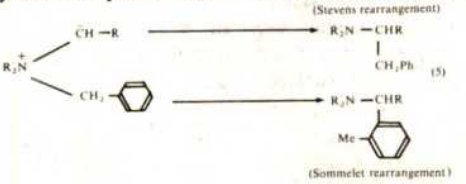
In the 1950s Wittig (and independently John D. Roberts) explored the synthetic and mechanistic aspects of benzyne. His studies were extended to other dehydroaromatics, dehydroheterocyclics and small-membered cycloalkynes in the 1960s. These short-lived intermediates were trapped as characteristic adducts with dienes or bases and this procedure led to novel syntheses. It may be an interesting aside that Wittig and Roberts believe that they are distantly related, as the name Dombrowski, occurred in both their families.

Much of Wittig's work was in the field of organic anionic chemistry. Here he carried out important investigations on metallated ethers (the Wittig rearrangement) in 1942



(4)

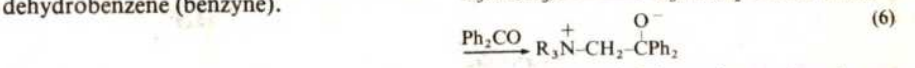
and ammonium ylides 2 years later



(5)

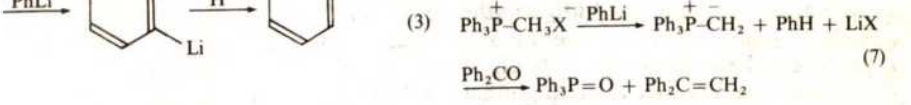


The zwitterionic ylides were obtained from the reaction of phenyl-lithium with quaternary ammonium halides and the unstable products converted with benzophenone to stable and easily characterised adducts.



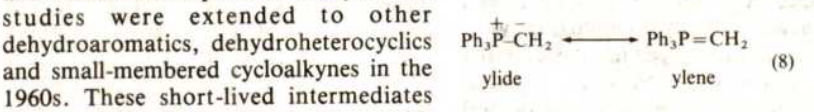
(6)

Extension of this work to phosphorus compounds led to the discovery of the phosphonium ylides in 1953, and their reactions with aldehydes or ketones to give the famous olefin synthesis which carries Wittig's name.



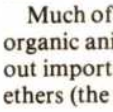
(7)

This ylide for which also the ylene canonical form is discussed



(8)

reacts *via* zwitterionic intermediates, usually unstable, but which can be isolated in special cases, to give olefins with a precisely defined location for the double bond.

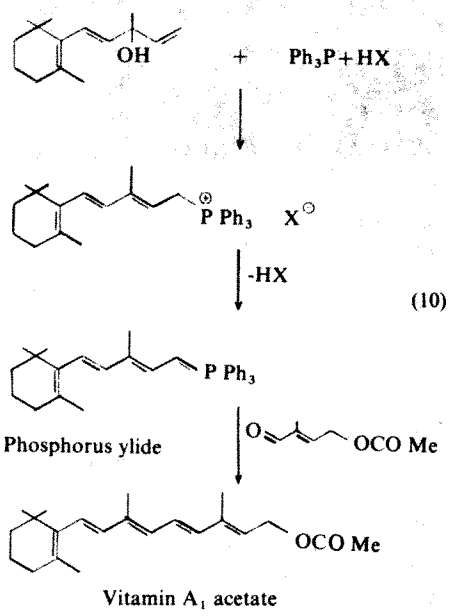


(9)

This olefin synthesis proceeds under mild conditions and is thus ideally suited for the synthesis of natural products. Recent work allows considerable control of whether *cis* or *trans* olefins are formed. Amongst the

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triumphs of this synthetic procedure are vitamin A_1, β-carotene, citranaxanthin (a food dye), vitamin D_2, progesterone, squalene, the sesquiterpenes α and β-sinensal (the aroma of orange oils), juvenile hormones (with potential in pest control), prostaglandin PGF_2, the Queen bee substance (a pheromone), an optical brightener (PALANIL^R Brilliant White R) for cotton and polyester fibres, and many more. In particular vitamin A_1 is now produced on a tonne scale by this method. The BASF process for its synthesis is outlined below.



Vitamin A_1 acetate

Another contribution by Wittig was the use of organolithium compounds and other strong bases to obtain carbenes in 1959, now widely recognised as important intermediates in many organic reactions. More recently carbenes have been observed in a variety of complex organometallic compounds, and some of these have been characterised by X-ray crystallography.

An α-carbon-hydrogen bond is essential for the formation of phosphorus ylides. Utilising the absence of such a structural feature in phenyl derivatives, Wittig synthesised pentaphenylphosphorane, Ph_5P.

$$Ph_4\overset{+}{P}\overset{-}{X} + PhLi \rightarrow Ph_5P + LiX \qquad (11)$$

By analogous routes pentaphenyl derivatives of arsenic, antimony and bismuth were obtained, as were tetraphenyl derivatives of sulphur, selenium and tellurium and a triphenyl derivative of iodine.

In addition to the above mentioned neutral organometallic compounds Wittig prepared numerous anionic derivatives, the 'ate'-complexes, and explored their chemistry. Two examples must suffice: sodium tetraphenylborate, $NaBPh_4$, used in the estimation of potassium and of some alkaloids, and lithium hexaphenylantimonate, $LiSbPh_6$.

Science may be impersonal — scientists certainly are not. So I cannot write about Georg Wittig the scientist without mentioning Georg Wittig the man. My first contact with Wittig was when, as a PhD student I wrote to him about a paper of his and Vidal's on the hydrocarbon fluorene and received a most courteous reply. I well recall the kindness with which he later received me and many other young scientists at his Institute in Heidelberg and his and his wife's generous hospitality. His Institute mirrored his personality, courteous and always kind, and was not one where continuous internecine warfare was current: his kindness made him an exceptionally effective teacher to those fortunate enough to have been his pupils. He was an inspiration to all those who came in contact with him.

His wife Waltraut, herself a PhD in chemistry, was his constant and trusted companion and her unexpected death last year was a severe blow to him. With firmness and kindness she stood by his side and shielded him from most of the more mundane cares of modern life leaving him free to concentrate on his scientific work. It must be a great sadness to him, as well as to those of us who knew her, that she did not live to see his work rewarded with the award of a Nobel Prize.

Georg Wittig's lasting achievements speak for themselves but two aspects deserve particular comment. Most of his work was done without the benefit of modern high technology instruments such as high field NMR spectrometers, four-circle diffractometer and so on. His most important 'instruments' were his vast chemical knowledge, his fine intellect and a superb chemical intuition. And much of his best work was done at an age when for many others the spring of scientific innovation has dried up. Good wine matures with age and Georg Wittig is undoubtedly of the best vintage. □

Robert A. Shaw is Professor of Chemistry at Birkbeck College, University of London.

Protean muscle

from Alan Weeds

MUSCLE is a highly adaptable and responsive tissue. Its physiological and biochemical properties accommodate to changes in functional requirements, altered patterns of nerve stimulus and hormonal variation. Dramatic changes are seen in the pattern of gene expression during embryological development and following denervation or local injury: such systems provide valuable models for unravelling the temporal sequence of these genetic events and elucidating their ontogeny. To what extent does the pattern of nerve impulses control muscle performance? How is this modified by use, hormones and other nutritional factors? What is the mechanism whereby these external influences affect the genetic programme of muscle? Is there a common messenger? These were some of the questions discussed at a recent meeting on Muscle Plasticity*.

Myosin changes during development

Probably the most controversial aspect of the meeting concerned the nature of myosin gene expression in developing muscle, a subject that has been debated in *Nature* recently (see **280**, 321; 1979). G. Gauthier (University of Massachusetts, Worcester) described the use of purified antibodies to fast and slow chicken muscle myosins to identify cross-reacting species in developing rat muscles by indirect immunofluorescence. In early post-natal development all fibres of extensor digitorum longus (EDL, which is fast-twitch in the adult) and soleus (slow-twitch in the adult) stained with both antibody preparations. Segregation of staining into the adult pattern occurred only later, probably coincident with the stage when the muscles cease to be polyneuronally innervated and are stimulated through a single axon. The experiments suggest the presence of both adult forms of myosin within the same fibre or may indicate a different myosin isoenzyme which cross reacts with both sets of specific antibodies. By contrast, N. Rubinstein (University of Pennsylvania, Philadelphia) argued that most of the fibres of both EDL and soleus muscles from 18-day rat embryos stained exclusively with antibodies to fast muscle myosin while only a few stained with antibodies to slow muscle myosin. At birth all the fibres still reacted with anti-fast myosin antibodies, but the level of staining was weaker in those fibres which also reacted with anti-slow myosin antibodies. These experiments, combined with two-dimensional gel electrophoresis of the myosin light chains suggest that only fast muscle myosin is synthesised in both fast and slow muscles at the earliest stages of development. Cultures derived from chick pectoralis and anterior latissimus dorsi muscles *in vitro* (fast and slow respectively) were also shown to synthesise only fast myosin by these criteria.

R. Whalen (Institut Pasteur, Paris) reported evidence for an embryonic form of myosin in developing rat muscles and also in bovine and chicken muscles. This was based on light chain patterns in two-dimensional gel electrophoresis and also on

*The International Symposium on Plasticity of Muscle was held at the University of Konstanz, Germany between 23 and 28 September and organised by Professor D. Pette. The proceedings of the meeting will be published in early 1980 by Walter de Gruyter, Berlin.

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gel fingerprints of chymotryptic fragments of the myosin heavy chains. Embryonic myosin is the predominant species in primary tissue culture cells after fusion and is present in newborn rat muscles as a minor species. Thus in soleus muscle of newborn rats, all three forms of myosin occur. J. Hoh (University of Sydney) also provided evidence for an embryonic form of myosin based on gel electrophoresis of the native protein.

While differences in the immunofluorescence results may reflect different techniques and variations in the response of the animals used to raise the antibodies, these different results are at variance, particularly those of Rubinstein, and will require further investigation. Nevertheless, the evidence for an embryonic myosin is persuasive and it is also clear from a number of systems that two or more myosin isoenzymes may coexist within the same fibre. But we do not know whether, if these myosins are synthesised simultaneously, polyneuronal innervation is required or whether this is part of the intrinsic developmental genetic programme. Part of the problem is to establish the precise times at which polyneuronal innervation becomes effective and is subsequently eliminated. Further experiments on the sorting of both myosin and troponin isoforms (discussed by S. Perry, University of Birmingham) are needed to elaborate the complex sequence of myofibrillar gene expression during differentiation and development. E. Kugelberg (Karolinska Institut, Stockholm) showed that there was a steady transformation of fibre motor units from fast to slow in soleus muscles from maturing rats, but whether the intermediate contraction times result from changed discharge frequencies of the motoneurones remains to be proved.

Innervation and regeneration

A related problem, discussed by R. Bischoff (Washington University, St Louis) and A. Kelly (University of Pennsylvania, Philadelphia) concerned muscle regeneration following denervation or local injury. Bischoff suggested two routes. Satellite cells located beneath the basement membrane may proliferate and fuse to form new myotubes or, under conditions where these are killed, dedifferentiation of myofibres occurs, which subsequently form immature myotubes leading to new myofibrils. This demonstrates once again the remarkable versatility of muscle tissue. Kelly argued that the programme of myosin gene expression during regeneration mimicked that occurring during development.

G. Vrbová (University College, London) presented evidence that polyneuronal innervation disappears when the animal starts moving and suggested that retraction of nerve fibres from the endplate might be due to the action of proteolytic enzymes, stimulated by acetylcholine and calcium ions. Thus maturation of muscle fibres depends not only on stimulation by a single motoneurone but also on muscle use. Other speakers also emphasised the importance of use. D. Goldspink (Queen's University Belfast, Northern Ireland) reported that limb immobilisation produced a marked increase in protein degradation and simultaneous decrease in synthesis, but atrophy was greater in the normally more active soleus muscle than in EDL. Stretching the muscles reduced the rate of atrophy through increased protein synthesis. H. Vandenburgh (National Institute of Mental Health, Bethesda) demonstrated increased protein synthesis in cultured muscle cells in response to stretch. He suggested that stretch activates the sodium/potassium ATPase, which might enhance amino acid transport. J. Etlinger (SUNY Downstate Medical Center, Brooklyn) showed effects of calcium ions as well as stretch on protein degradation and biosynthesis in rat muscles. Release of myofilaments during degradation is stimulated in the presence of calcium ions by way of proteolysis. Thus the application of mechanical force influences ion movements in muscle membranes which may be responsible for the observed changes in protein turnover.

The effects of a number of hormones on protein synthesis and degradation were reviewed by A. Goldberg (Harvard Medical School, Boston), emphasising the dynamic nature of muscle as providing 80% of available body protein for metabolism. C. Ianuzzo (York University, Toronto) and A. Montgomery (Newcastle General Hospital, Newcastle upon Tyne) showed the effects of altered thyroid hormone levels on the presence of fast-twitch fibres in rat soleus muscles. Thyroid status clearly influences the pattern of myosin gene expression in addition to its general effects on protein turnover.

Electrical stimulation

The other major area of discussion centred on altered patterns of electrical stimulation and use in adult muscles. Although denervation, spinal transection and cross-reinnervation have provided useful models, these surgical procedures are often complicated and the effects long term. Emphasis at this meeting was on the use of chronic electrical stimulation to investigate the influence of different firing rates on speed of shortening and metabolic enzyme levels. T. Lømo (University of Oslo, Norway) reported chronic stimulation experiments on rat muscles following nerve section, where the only stimulus reaching the muscles was the externally applied one. His most interesting findings were that infrequent 100 Hz pulses applied to slow muscles produced properties characteristic of fast-twitch muscles. But he found that the speed of contraction was regulated independently from the muscle's endurance. O. Hudlická (University of Birmingham) described effects of chronic stimulation in increasing blood flow and oxidative enzyme levels, while D. Pette (University of Konstanz, Germany) showed dramatic changes in metabolic enzyme activities, especially hexokinase, within 24 hours following stimulation of rabbit fast-twitch muscles at 10 Hz for 12 hours per day. C. Heilmann (University of Konstanz) examined effects on the sarcoplasmic reticulum and found reduced calcium uptake and ATPase activity within 2 days of applying artificial stimulation. In addition there were changes in the composition of phosphorylated proteins in the reticulum membrane. S. Salmons (University of Birmingham) showed an increase in the activity of enzymes involved in polyamine synthesis of nearly two orders of magnitude within 28 hours of applying the external stimulator, which might reflect increased levels of mRNA processing or translation in these muscles. Although this model was discussed extensively, Vrbová suggested that pharmacological agents and hormones might prove more valuable for the future because of the 'insulting' effects of this abnormal form of stimulation on the muscles.

The meeting represented somewhat of a watershed. I left with the feeling that the groundwork has been laid but that there was much more to achieve. Particularly important would be tissue culture systems which were free from the vagaries of whole animal models. A. Shainberg (Bar-Ilan University, Ramat-Gan, Israel) introduced us to novel experiments analysing acetylcholine receptors on myotubes following electrical stimulation. Although the importance of the membrane became increasingly clear as the meeting proceeded, relatively little attention has been paid to it in the past. Whatever the signals may be that control the genetic programme, the membrane acts as their gate. Our mercurial muscles have much yet to reveal. □

Alan Weeds is a member of the scientific staff at the Medical Research Council Laboratory of Molecular Biology, Cambridge.

Life in a cold climate

from W. Nigel Bonner

THE Antarctic provides a testing environment for many forms of life. Severe climate, characterised by large temperature range and oscillations, with long periods of subzero temperatures, combine with extreme isolation to create a set of conditions where the critical response of an organism to its environment can often be simply expressed in terms of one or two key features. Studies of such responses allow the description of the adaptational strategy of certain polar organisms and are being undertaken by the British Antarctic

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Survey, (a component body of the Natural Environment Research Council). Some recent work was reported at a conference at the Linnean Society last month*.

The typical Cryptostigmatid mite, *Alaskozetes antarcticus,* which is an important component of the very sparse Antarctic terrestrial invertebrate fauna, is continually exposed to the risk of tissue freezing. Freezing is fatal to all stages of this mite. The risk of freezing is increased if the mites have been feeding, as food remains in the gut provide potential ice-nucleators when supercooled. W. Block and S.R. Young have found that *Alaskozetes* can to a great extent avoid freezing first by ceasing to feed in the autumn, so that the gut is emptied of potential ice-nucleating agents, and second by developing an ability to supercool to −26°C. Supercooling is enhanced by glycerol, which the mite can synthesise as a response to low temperature and low relative humidities, to allow survival to at least −31°C. Juvenile mites possess an even greater ability to tolerate cold than do adults. In this way juveniles and adults avoid tissue freezing in winter in their maritime Antarctic habitat. *Alaskozetes* maintains a metabolic rate some two to three times higher than that of comparable temperate mites over the range 0 to +10 °C. This elevation of metabolism or cold adaptation combined with its strategy for avoiding freezing allows large populations of mixed instars to survive the Antarctic winter and so use the short summer period for growth and reproduction. Life cycles of such species are necessarily long (up to 2-3 years) in the Antarctic.

Conditions are very different in the Antarctic sea. Here temperatures, although low, are stable in comparison with the terrestrial environment and freezing is a problem for relatively few organisms (notably fish and some shallow-water molluscs). More important is the great seasonal variation in food availability, caused by changes in light-climate and ice-cover. This results in a brief season of phytoplankton bloom, in which food is abundant, followed by a long period of scarce or erratic supply. Andrew Clarke suggested that Antarctic marine invertebrates, unlike *Alaskozetes,* are characterised by low basal metabolic rates, (regarding basal metabolism as the generation of ATP for protein turnover, for membrane pumps, and (with NADPH) for lipid turnover, plus the cost of circulation). Coupled with low basal metabolic rates are slow growth, reduced annual reproductive effort and by analogy with fish, low rates of protein synthesis. Together, these result in a reduction of the total food requirement, thus facilitating survival in an environment when food is limited for a large part of the year.

The absence of planktonic larval stages of benthic invertebrates is characteristic of the polar seas. In Antarctic prosobranch molluscs, for example, most forms produce a few large yolky eggs from which tiny but perfect snails emerge after a long development. This parallels the principal reproductive strategy of other polar benthic invertebrates and G.B. Picken argued that this style of early development, whether by means of egg capsules, brood protection or viviparity, has strong survival value in localities where low temperature and slow growth mean that planktonic larvae would be unable to exploit the brief abundance of phytoplankton during the summer bloom. The trend towards reduced fecundity, enhancing the survival of the large, well-developed juveniles in comparison with more numerous but smaller planktotrophic larvae, and the extreme isolation of shallow-water benthic habitats in the Antarctic, may be the principal factors responsible for the high level of species endemism found there.

Sea-birds also exploit the Antarctic environment. Being homeotherms, low temperatures are not primarily a problem for them, provided energy sources are adequate. During the breeding season, however, which is in general timed to take advantage of the high seasonal food availability, food demand is dramatically increased at those sites where the birds concentrate. South Georgia is such a site and J.P. Croxall and P.A. Prince have studied how the various species breeding there divide the food resources between them. In some cases chick-feeding periods are complementary between species pairs, so that direct competition for food between similar feeders is reduced. In others, there may be species differences in the frequency with which chicks are fed, allowing one species to exploit more distant feeding grounds than another. Most commonly, however, differences are found in the detailed composition of diets. The two very similar albatrosses, the Black-browed Albatross and the Grey-headed Albatross, for example, have as principal components of their diet krill and squid respectively. Because squid is less nutritious than krill Grey-headed Albatrosses rear their chicks more slowly and are unable to breed every year. However, in compensation their adult survival is substantially greater than in the Black-browed Albatross. □



Elephant Seal breeding beach. Studies on Elephant Seals at South Georgia by British Antarctic Survey have shown how this species has recovered from the effects of commercial sealing in the past. (Photograph by T.S. McCann.)



Terrestrial mites at Signy Island, maritime antarctic. Adults, nymphal stages and eggs of the detritivore. *Alaskozetes antarcticus* are seen on a rock surface with four individuals of the lighter-coloured predator *Gamasellus racovitza.* (Photograph by I.B. Collinge, BAS.)

*Nine papers reporting recent biological research in the Antarctic by British Antarctic Survey were presented at a symposium at the Linnean Society on 11 October, 1979. The proceedings of the symposium will be published as Vol. 14 (part 1) of the *Biological Journal of the Linnean Society.*

W. Nigel Bonner is Head of the Life Sciences Division, British Antarctic Survey.

James Clerk-Maxwell—100 years later

Cyril Domb

King's College, University of London, Strand, London WC2, UK

This year is the centenary of the death of James Clerk-Maxwell, whose theory of the electromagnetic field provided the background essential to the development of Einstein's ideas. Einstein, at the commemoration of the centenary of Maxwell's birth in 1931, described the change in conception of reality in physics after Maxwell as "the most fruitful that physics has experienced since the time of Newton".

THE concept of electromagnetic radiation originated with Maxwell, and his field equations paved the way for the special theory of relativity. Newton, Maxwell and Einstein are in a class of their own among physicists, and it is remarkable that Einstein was born in the year that Maxwell died, as in many respects Einstein took over where Maxwell left off. The quantum theory also leaned heavily on Maxwell's ideas. Max Planck introduced his quantum hypothesis to account for the spectrum of 'black-body radiation' (electromagnetic radiation in thermal equilibrium with matter at a given temperature) and to resolve the UV catastrophe of the classical theory for which an infinite amount of energy was present in radiation of short wavelengths. In the subsequent development of the theory of the structure of atoms and molecules and of matter in general, the interaction between matter and radiation has a central role.

Maxwell also made fundamental contributions to other branches of physics, notably thermodynamics, the kinetic theory of gases (the idea of applying probability and statistics to molecules is due to Maxwell), colour vision and colour photography. In the nineteenth century there was no serious division between theoretical and experimental physics. Maxwell engaged in both throughout his life, and his best known appointment is that of first Professor of Experimental Physics in the University of Cambridge.

Contemporary assessment

Maxwell's contemporaries did not think quite so highly of him. When the Cavendish Laboratory was initiated in 1871 Maxwell was only the third of those proposed for the appointment of director. The first was William Thomson (later Lord Kelvin). He did not wish to move from Glasgow and suggested Helmholtz who also refused to move from Berlin where he had just been appointed director of a new laboratory. Only then was Maxwell approached, and the letter of invitation by the Reverend E. W. Blore stated: "It has I believe been ascertained that Sir W. Thomson would not accept the Professorship. I mention this in case you should wish to avoid the possibility of coming into the field against him." When Maxwell did eventually take up the appointment at Cambridge the average attendance at his lectures was between two and three students!

His first academic teaching appointment was at Marischal College in the University of Aberdeen in 1856. In 1860 a merger took place between the two constituents of this University, King's College and Marischal College, and one of the two professors of Natural Philosophy became redundant; David Thomson of King's College was allowed to continue, ostensibly because of superior teaching ability, and Maxwell was declared redundant.

Maxwell applied for a vacancy at the University of Edinburgh but he was turned down in favour of his school friend Peter Guthrie Tait who was again considered a better teacher. Fortunately King's College London appointed him to the Chair of Natural Philosophy. The five subsequent years were undoubtedly the most fruitful of his career. He was elected to the Royal Society in 1861 aged 29. His two classic papers on the electromagnetic field were published in 1862 and 1864. In 1861 he delivered a lecture at the Royal Institution on the theory of three primary colours, and demonstrated the first colour photograph. The theoretical and experimental work on the viscosity of gases which took place during these years culminated in his Bakerian lecture to the Royal Society in 1866. He supervised the experimental determination of electrical units for the British Association's Committee; this work of measurement and standardisation led to the establishment of the National Physical Laboratory. He also measured the ratio of electromagnetic and electrostatic units of electricity, and confirmed that it was in satisfactory agreement with the velocity of light as predicted by his theory.

But even the association with King's College was described in negative terms by the College historian. Maxwell resigned his Chair in 1865 and retired to the family estate in Glenlair, Scotland. When the centenary history of the College was published in 1928 it claimed that Maxwell was asked by the Governors to resign because he could not keep order at lectures. There is, however, no hint of such a situation in the biography by Campbell, in the obituary notice in the Royal Society or in any of Maxwell's correspondence. I have recently investigated this claim and found it devoid of any substantial basis.

Maxwell emerged from retirement to take up the appointment at Cambridge in 1871, where he spent his final eight years; he died on 5 November 1879 aged only 48. He published just over 100 papers, received no public honours, and was buried quietly in a small churchyard in the village of Parton, Scotland. By contrast, his friend William Thomson lived on for nearly 30 years more, published 600 papers, received every possible honour and distinction, and was buried with great acclaim in Westminster Abbey.

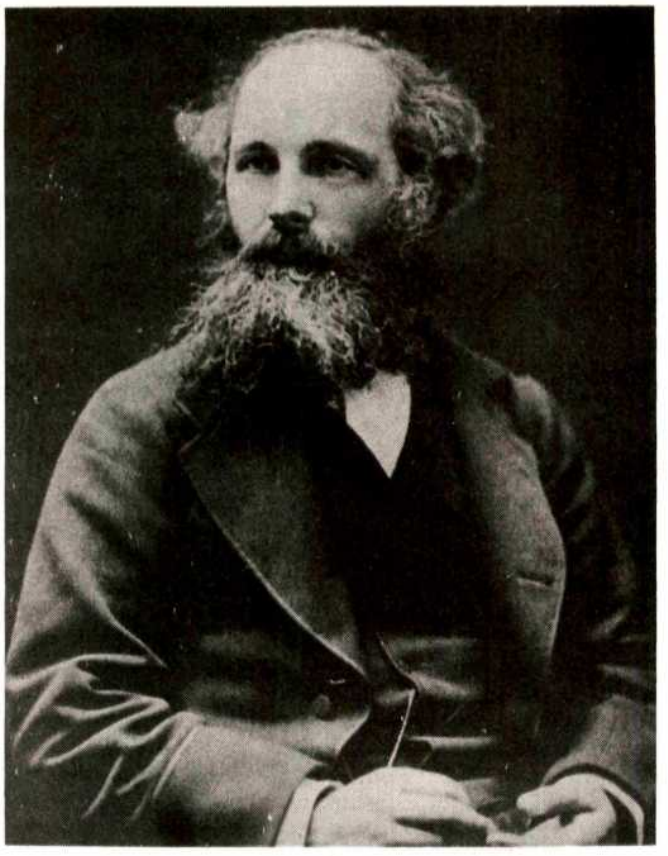
The true quality of Maxwell's genius became apparent only with the development of twentieth century physics. In addition to the major contributions to science mentioned previously, several ideas which he put forward quite casually have proved extremely fertile, and have led to important advances.

Biographical notes

Fortunately Maxwell's lifelong friend, Lewis Campbell (together with the first Demonstrator in Physics at the Cavendish Laboratory, William Garnett) produced a definitive biography within a few years of his death. Other biographical essays have appeared, the most important of which (by C. W. F. Everitt) provides new biographical material, and reviews

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From a portrait at King's College, London

James Clerk-Maxwell in his later years.

Maxwell's scientific achievements from the vantage point of the present day.

James Clerk-Maxwell came from a comfortable middle-class background. The original family name was Clerk, the additional surname Maxwell being added by his father because of the inheritance of the Middlebie estate from ancestors of that name. James, an only child, was born in Edinburgh where his father was a lawyer; his parents had married late in life and his mother was 40 years old at his birth. Shortly afterwards the family moved to Glenlair, the country house on the Middlebie estate.

James showed remarkable curiosity at an early age, and before he was 3 his mother wrote "he has great work with door locks, keys, etc. and 'show me how it doos' is never out of his mouth. He also investigates the hidden course of streams and bell wires. . . . As to the bells, they will not rest; he stands sentry in the kitchen, or he rings, and sends Bessy to see and shout to let him know, and he drags papa all over to show him the holes where the wires go through." He also had a phenomenal memory, and at the age of 8 he knew by heart the longest chapter of the Old Testament (Psalm 119 containing 176 verses).

Alas, his mother died in 1839 from abdominal cancer, the same disease to which he was to succumb at exactly the same age. A dull and uninspired tutor was engaged who claimed that James was slow at learning. Fortunately he was rescued by his aunt Jane Cay and was sent to school at the Edinburgh Academy. Among the other pupils were his biographer Lewis Campbell, and his friend Peter Guthrie Tait.

Maxwell's interests ranged far beyond the school syllabus, and he did not pay particular attention to examination performance. But he published his first scientific paper at the age of 14 describing a generalised series of oval curves which could be traced with pins and thread by analogy with an ellipse. This fascination for geometry and for mechanical models continued through his whole career, and was of great help in his subsequent research.

At the age of 16 he entered Edinburgh University where he read voraciously on all subjects, and published two more scientific papers. He went on to Cambridge in 1850, and here there is evidence that his exceptional powers began to be recognised. H. M. Butler (later Master of Trinity) wrote "When I came up to Trinity Maxwell was just beginning his second year. His position among us was unique. He was the one acknowledged man of genius among the undergraduates." Another friend W. N. Lawson wrote in his diary "Maxwell as usual showed himself acquainted with every subject on which the conversation turned. I never met a man like him. I do believe there is not a single subject on which he cannot talk, and talk well too, displaying always the most curious and out-of-the-way information."

A more discriminating appraisal came from his mathematics teacher, William Hopkins, the famous 'wrangler maker' of the period whose students included G. G. Stokes, William Thomson, Arthur Cayley, P. G. Tait and E. J. Routh. Of Maxwell he is reported to have said that he was the most extraordinary man he had met with in the whole course of experience; that it seemed impossible for him to think wrongly on any physical subject, but that in analysis he was far more deficient (other contemporaries testified to Maxwell's preference for geometrical over analytical methods). This shrewd assessment was borne out later by several important formulae advanced by Maxwell where the results have proved to be correct but the mathematical arguments are faulty.

In 1854 Maxwell was second wrangler and first Smith's prizeman; he was elected to a Fellowship at Trinity, but his father's health was deteriorating, and he wished to return to Scotland. In 1856 he was appointed to the Chair of Natural Philosophy at Marischal College Aberdeen; but unfortunately before the appointment was announced his father died. This was a great personal loss; he had a close relationship with his father, they had corresponded almost daily, and had always spent vacations together.

Maxwell must now have felt the need for his own family life, and in June 1958 he married Katherine Mary Dewar, daughter of the Principal of Marischal College. The union was childless, and is described as a "married life . . . of unexampled devotion". When Maxwell was away from home he would write to his wife every day, sometimes even twice a day, giving all details of his activities. When his wife was ill during their stay in Cambridge he did not sleep in a bed for three weeks. Reciprocally when Maxwell had an attack of smallpox at Glenlair in September 1860 his wife was left quite alone with him—the servants coming only to the door of the sideroom.

Later biographers have been less sympathetic to Mrs Maxwell. However, Everitt points out that the suggestion repeated by many writers (including Sir Edmund Whittaker) that she attempted to interfere with his scientific work, emanated from Mrs Tait, and should be completely discounted. For example, his wife helped him devotedly in his experiments on colour vision and the viscosity of gases.

Maxwell moved from Aberdeen to London in 1860. He took up residence in Kensington where he stayed until 1866. It was here that an experimental test was undertaken of his theoretical prediction that the viscosity of a gas should be independent of its density, his wife acting as stoker. He carried out many other experiments in a large attic which ran the whole length of the house. When he experimented at the window with the colour-box used for studies of colour vision (painted black, and nearly eight feet long) his neighbours thought him crazy to spend so many hours staring into a coffin.

After Maxwell retired to Glenlair, he continued to visit London every spring, and served as external examiner for the Mathematics Tripos at Cambridge. In the spring and early summer of 1867 he toured Italy; he had a good knowledge of French, German and Italian, the only language which gave him any difficulty being Dutch. But most of his energy during this period was devoted to writing his famous treatise on electricity and magnetism.

In 1871 when he was approached regarding the new Chair at Cambridge, Maxwell showed no enthusiasm for the position. In his reply to the Reverend E. W. Blore he said "I had no intention of applying for the post when I got your letter, and I have none now, unless I come to see that I can do some good by it." But after persuasion by G. G. Stokes, Lord Rayleigh and others he allowed his name to go forward and was duly elected.

He set about designing and superintending the erection of the Cavendish Laboratory. The building was not completed until 1874, and was not furnished with proper apparatus until 1877. Maxwell had few students, but they were of the highest calibre, and included W. D. Niven, Ambrose Fleming, R. T. Glazebrook, J. H. Poynting and Arthur Schuster.

At Cambridge Maxwell spent a great deal of time and effort editing the unpublished manuscripts of Henry Cavendish (who had donated the funds for the establishment of the Cavendish



The house which the Maxwells occupied during their stay in London (1860–66). The address was then 8 Palace Gardens Terrace, Kensington, but in 1869 the number was changed to 16. Experiments on colour vision and viscosity of gases, in which he was helped by his wife, took place in the attic.

Laboratory). Maxwell did a superb job, repeating key experiments, and establishing priority for Cavendish in the discovery of the inverse square law of electrostatics. But it cost all the spare time of the last five years of his short life, and surely his genius could have been put to more profitable use.

In the Easter term of 1879 Maxwell was ill on several occasions; he returned to Glenlair in June but his condition did not improve. On 2 October he was told that he had no more than one month to live. The religious faith which had characterised his whole life became even more manifest during his last few weeks. He returned to Cambridge, and expressed particular concern that proper arrangements should be made to look after his wife whose health had been poor. His mind remained clear to the end; in the words of Dr Paget who attended him "No man ever met death more consciously or more calmly".

Major contributions to science

Electromagnetism: It is Maxwell's researches on electromagnetism which have classified him with the great scientists of history. Maxwell always acknowledged his debt to Faraday, and in the preface to his *Treatise on Electricity and Magnetism* says that his major task was to convert Faraday's physical ideas into mathematical form, and hence to make them more widely accessible.

ON PHYSICAL LINES OF FORCE.

The velocity of light in air, as determined by M. Fizeau*, is 70,843 leagues per second (25 leagues to a degree) which gives

$$V = 314,858,000,000 \text{ millimetres}$$

$$= 195,647 \text{ miles per second} \quad \ldots\ldots\ldots\ldots\ldots\ldots (137).$$

The velocity of transverse undulations in our hypothetical medium, calculated from the electro-magnetic experiments of MM. Kohlrausch and Weber, agrees so exactly with the velocity of light calculated from the optical experiments of M. Fizeau, that we can scarcely avoid the inference that *light consists in the transverse undulations of the same medium which is the cause of electric and magnetic phenomena.*

Cambridge University Library

Extract from Maxwell's 1862 paper in the *Philosophical Magazine* in which he first formulated the electromagnetic theory of light.

His original contributions to electromagnetism are contained in three large sets of papers. The first, "On Faraday's Lines of Force", was published in 1856 during his first stay at Cambridge. Its aim was to derive all the standard results of electricity and magnetism using the 'medium' concept rather than that of action at a distance. The second, "On Physical Lines of Force", appeared in 1861–62. In attempting to illustrate Faraday's law of induction (that a changing magnetic field gives rise to an induced e.m.f.) Maxwell constructed a mechanical model and found that there would be a corresponding 'displacement current' in the dielectric medium. This medium could then be the seat of transverse waves, and on calculating the velocity of these waves he found that they were very close to the velocity of light. He concluded dramatically "we can scarcely avoid the inference that light consists in the transverse undulations of the same medium which is the cause of electric and magnetic phenomena". The final paper "A Dynamical Theory of the Electromagnetic Field", published in 1864, dispensed with the mechanical model, and formulated the field equations in the way in which they have been used subsequently.

Colour vision: To understand the theory of colour vision it is important to differentiate between the optical (or true) spectrum of a beam of light and the chromatic spectrum as seen by the eye. Beams of light with different optical spectra can have the same chromatic spectrum. Isaac Newton had shown that the optical spectrum of white light is very complex, but Thomas Young at the beginning of the nineteenth century proposed that any chromatic spectrum can always be analysed into three components corresponding to three receptors in the eye.

Maxwell's work on colour vision started when he was an undergraduate at Edinburgh and continued for many years afterwards. He chose red, green and blue as primary colours, and devised a colour top by means of which tinted papers of different colours could be mixed in varying intensities and the results determined. In this way he initiated 'quantitative colorimetry', analysing every hue into its appropriate components. Maxwell made the important observation that Young's hypothesis works only if negative as well as positive contributions are allowed. He also suggested that colour blindness corresponds to the absence of a red-sensitive receptor, and devised a pair of spectacles to enable a colour blind person to differentiate between red and green.

He invented a colour box in which beams of light of different colours could be mixed in variable proportions to match any given hue. With this apparatus he was able to achieve great accuracy in his experiments to chart the colour response of

different individuals, and to demonstrate that the colour discrimination of normal people is very good.

Colour photography: From the three-colour theory Maxwell concluded that by photographing through filters of three different colours, and then recombining the images, it should be possible to make a colour photograph of any object. He demonstrated this practically in a lecture to the Royal Institution in 1861 by photographing a tartan ribbon. This demonstration should not have worked, since the photographic emulsions available to Maxwell were not red-sensitive. The mystery was solved when a group of Kodak workers repeated Maxwell's procedure exactly, and found that the red filter also passed UV, and the red in the ribbon apparently also reflected UV.

Saturn's rings: Whilst at Aberdeen Maxwell was able to demonstrate his mastery of classical physics in his Adams Prize Essay on Saturn's Rings, described by George Airy as one of the most remarkable applications of mathematics he had ever seen. Three alternative hypotheses were advanced for the constitution of Saturn's rings: (1) that the rings are solid, (2) that they are fluid or in part uniform, (3) that they consist of masses of matter not mutually coherent; it was necessary to examine for each hypothesis whether the conditions of stability are satisfied by the mutual attractions and motions of the planet and the rings. Maxwell concluded that the third hypothesis was the only one compatible with the stability of the rings. His investigation of this hypothesis laid the foundation for his subsequent work on the kinetic theory of gases.

Kinetic theory of gases

At the British Association meeting in Aberdeen in 1859 Maxwell pointed out that the velocities of molecules in a gas must follow a statistical distribution. Previously it had been naively assumed that they all had the same velocity. Maxwell derived a formula for this distribution at a given temperature and density, which has come to be known as the Maxwellian distribution. Although his derivation contained unwarranted assumptions, his result is valid and represents one of the most widely used formulae in statistical mechanics.

In later papers Maxwell investigated the transport properties of gases—the behaviour of viscosity, thermal conductivity and diffusion with change of temperature and pressure. He concluded that the viscosity of a gas should be independent of pressure, and found this result in reasonable agreement with experiment. He noted a particular simplification which occurs for molecules interacting with a repulsive force inversely proportional to the fifth power of their distance apart. In the last major paper of his life, published in 1879, he laid the foundations of the theory of rarefied gas dynamics.

Fertile ideas

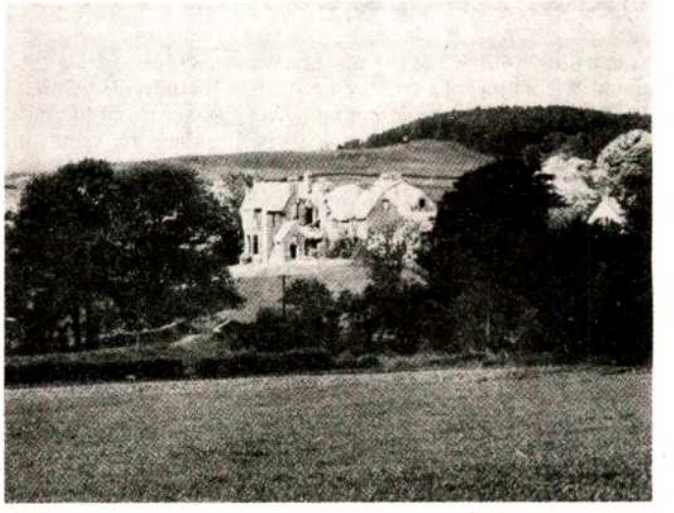
Several casual ideas of Maxwell have led to important developments. Perhaps the best known is the Maxwell Demon which he introduced near the end of his text-book *Theory of Heat*, published in 1871, to demonstrate the statistical character of the second law of thermodynamics. The demon was a hypothetical being who could follow the path of every molecule, and seated on a trapdoor between two vessels A and B, would open and close the door so as to allow fast molecules to go from A to B and slow molecules from B to A. Hence the temperature of B would be increased and A decreased without expenditure of work thereby contravening the second law of thermodynamics. The suggestion intrigued many distinguished scientists subsequently. If one could devise a practical realisation of the demon, the consequences could be very important. Clearly the demon's intelligence must be related in some way to entropy, and this correlation was given a mathematical formulation in Brillouin's theory of information which thereby demonstrated that the demon could not operate. Brillouin's interpretation of information as negative entropy, which was responsible for exorcising the demon, is a key concept in present understanding of the thermodynamics of biological systems.

In 1868, Maxwell provided the first analytical treatment of governors, devices which ensure that the velocity of the machine remains nearly uniform despite variations in driving power or resistance. Devices of this kind had been used by James Watt (to control his steam engine), James Thomson, C. W. Siemens, M. Foucault and others. Maxwell derived differential equations for the motion, introducing a term later to be characterised as negative feedback. He solved the equations, and determined conditions for the motion to be stable, and the governor to function. His paper laid the foundation of control theory, or cybernetics.

The Bakerian Lecture to the Royal Society in 1869 was delivered by Thomas Andrews, and described the classic experiments on carbon dioxide from which he derived the relationship between the liquid and gaseous states. Four years later Johannes van der Waals presented his thesis in which, starting from a plausible molecular hypothesis, he derived his equation of state for fluids. Maxwell was very impressed by this thesis, and made the effort of learning Dutch to be able to study it. He reviewed it in *Nature* in 1874, and in a lecture to the Chemical Society in 1875, where he supplied a crucial step missing in the van der Waals treatment. The argument which Maxwell used to justify his construction was heuristic rather than rigorous; it was only in the 1960s that this result was shown to be rigorously correct.

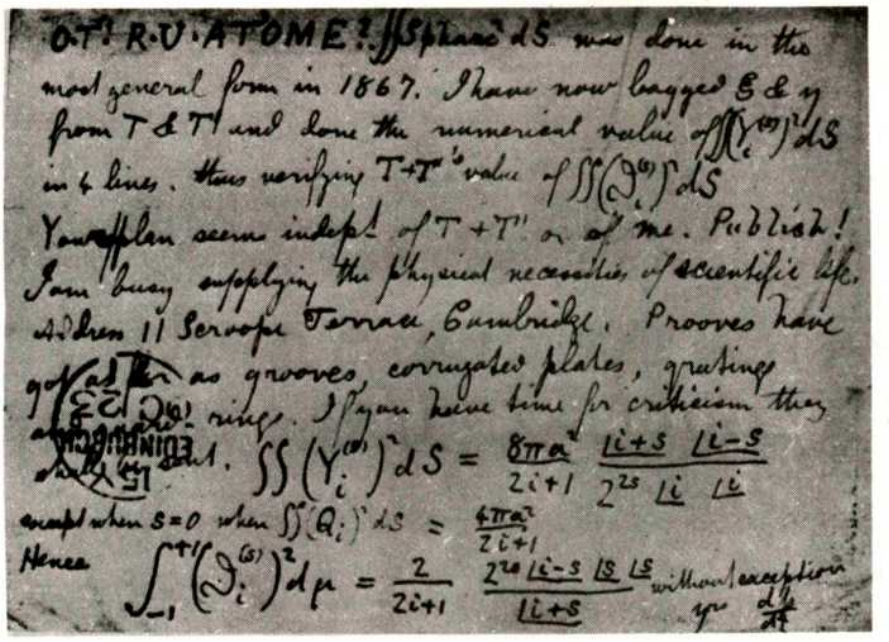
Whilst still a student at Cambridge in 1853 Maxwell undertook a study in geometrical optics "suggested by the contemplation of the structure of the crystalline lens in fish". He looked for a medium in which focusing would be perfect—all rays proceeding from any point in the medium would meet accurately in another point; and using a method deduced by analogy from Newton's *Principia* he arrived at a mathematical description of the medium. Nearly a century elapsed before Rudolf Luneberg revived the subject, and found other instances of perfect imaging devices.

A striking example of the casual way in which Maxwell produced original ideas is provided by the theory of propagation of light in a dispersive medium. A formula for the refractive index was put forward by Sellmeyer in 1871. Twenty years after Maxwell's death, Lord Rayleigh published a note in the *Philosophical Magazine* pointing out that Maxwell had considered the question before anyone else. But Sellmeyer could hardly have been expected to know of Maxwell's work as it was contained in a question set for the Mathematical Tripos examination on 21 January 1869.



Photographed by R. A. R. Tricker

Glenlair, the country house which Maxwell inherited and to which he moved when he resigned from King's College in 1865. He left in 1871 to become the first Cavendish professor at Cambridge. (The interior of the house was destroyed by fire in 1929.)



Cambridge University Library

One of the postcards sent by Maxwell to his friend Peter Guthrie Tait. William Thomson (Lord Kelvin) is referred to as T, P. G. Tait as T', and Maxwell signs himself dp/dT in accordance with the thermodynamic relation dp/dT = JCM. The term T & T' used in lines 3 and 4 refers to the famous nineteenth century text by Thomson and Tait, *Elements of Natural Philosophy*.

Maxwell and Einstein

Maxwell regarded the ether as the seat of electromagnetic waves, and after developing his electromagnetic theory, he devoted much thought to the problem of the 'ether drift' or how much the velocity of light would be changed by the motion of the emitter. He convinced himself that in a terrestrial experiment it was necessary to measure the time which has elapsed in a go and return journey and the effect would be of the order of v^2/c^2 (where v is the velocity of the emitter and c the velocity of light) which would be too small to measure. But in the last year of his life he wrote to the astronomer D. P. Todd suggesting that he look for a change in the periodicity of the satellites of Jupiter as the planet changed its position relative to the Earth; this periodicity should depend on the ether velocity along a line joining the Earth and the planet. These considerations were an important stimulus to the famous Michelson–Morley experiment which played such a crucial role in Einstein's development of special relativity.

Maxwell maintained a close scientific friendship with William Thomson (later Lord Kelvin) and Peter Guthrie Tait. For many years the three exchanged postcards, the former being referred to as T and the latter as T', and Maxwell as dp/dT (this was based on the thermodynamic relation dp/dT = JCM giving Maxwell's initials, where p is the pressure, T the temperature, J Joule's constant, C Carnot's function T^{-1}, and M the rate of increase of heat in an isothermal change of volume). One of the most influential text books of the period was Thomson and Tait's *Treatise on Natural Philosophy*. When Tait sent a preliminary draft to Maxwell he wrote back criticising them for confusing mass and weight: "You may place the two masses in a common balance (which proves their weight equal), you may then cause the whole machine to move up or down. If the arm of the balance moves parallel to itself the *masses* must also be equal."

The identity of mass-like and weight-like properties of matter is a coincidence in Newton's theory, and constituted one of Einstein's major criticisms of the theory.

In his classic 1864 paper "A Dynamical Theory of the Electromagnetic Field" Maxwell tried to consider the formulation of a field theory of gravity which also satisfies an inverse square law of force. But the change of sign in the interaction and the requirement that energy must be positive led to the conclusion that an enormous intrinsic energy must be associated with the medium. "As I am unable to understand in what way a medium can possess such properties I cannot go further in this direction in searching for the cause of gravitation." Maxwell clearly wanted to go in the Einstein direction, but the necessary basic equipment was lacking.

Finally in a lecture delivered to the Chemical Society in 1875 Maxwell calculated the specific heat of a gas of molecules with n degrees of freedom. He found that the ratio γ of C_p the specific heat at constant pressure, to C_v the specific heat at constant volume, should be of the form $(2+n+e)/(n+e)$, where e is a positive quantity of unknown value relating to the stored energy of the molecule. For a monatomic molecule $n=3$, $e=0$ (no stored energy), $\gamma = 5/3$, a value which agrees well with experiment. For a diatomic molecule $n=6$, and the largest possible value of γ (corresponding to $e=0$) is 1.33; but the experimental values for several gases are found to be 1.408. Maxwell continued "I have now put before you what I consider to be the greatest difficulty yet encountered by the molecular theory".

The difficulty was resolved only by Planck's quantum hypothesis; and it was Einstein who first applied the hypothesis to the calculation of specific heats, and showed that the energy of an oscillator became frozen at sufficiently low temperatures.

Maxwell's versatility

This record by no means exhausts Maxwell's contributions to science and to general culture. This would require a much larger and more comprehensive article. He showed considerable ability as a poet, particularly for semi-humorous verse on scientific themes. He was concerned with scientific terminology, for example, coining the term curl which is now used almost universally in vector analysis. Amongst other scientific innovations he produced a theorem on reciprocal diagrams in frameworks which he later extended to continuous media, and which was of importance in the development of ideas on strength of materials. He devoted much time and effort trying to popularise the Gibbs approach to thermodynamics.

Maxwell's papers are full of novelty and interest. In 1854 he discusses what happens when a slip of paper falls through the air, noting that its general path is not in the vertical direction but inclined to it at an angle which remains nearly constant, its fluttering appearance due to a rapid rotation around a horizontal axis. He then advances reasons for this behaviour.

In the words of the late Charles Coulson "there is scarcely a single topic that he touched upon which he did not change almost beyond recognition".

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articles

An absorption feature in the spectrum of the pulsed hard X-ray flux from 4U0115+63

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A spectral feature, apparently an absorption line, has been observed at an energy of 20.1 ± 0.5 keV in the pulsed flux of 3.61 s X-ray pulsar 4U0115+63 using the UCSD/MIT instrument on HEAO 1. The line strength, expressed as equivalent width, is 3.1 ± 0.5 keV. Although essentially unresolved, the feature has a depth more than 60% of the continuum flux. If the feature arises by cyclotron resonance absorption near the magnetic poles of the neutron star, it implies a magnetic field of between ≈1.8 and ≈2.5 × 10^{12} G depending on the gravitational redshift (≲5–40%).

ALTHOUGH standard models (see ref. 1) of the pulsating binary X-ray sources as rotating, accreting magnetised neutron stars require magnetic fields B of the order of 10^{12}–10^{13} G, the discovery by Trümper *et al.*[2] of a strong pulsed 58 keV feature in 1976 and 1977 balloon observations of the spectrum of Her X-1 raised the important possibility of direct measurements of B. Trümper *et al.*[2] have argued that the feature they observed may result, as suggested earlier on theoretical grounds by Basko and Sunyaev[3], from cyclotron emission quantised in a field of ≈5.3 × 10^{12} G (uncorrected for redshift). The UCSD/MIT experiment on HEAO 1 confirmed the existence of this feature in the spectrum of Her X-1 on several occasions in 1978 (refs 4, 5).

The 1978 outburst[6,7] of 4U0115+63 led to discovery of its 3.61 s pulsations[6–8], accurate measurement of its position[9,10], and discovery of its binary nature[11], 24-d orbit[12], and 15th mag B-type optical counterpart[13], as well as the determination of X-ray spectra and pulse profiles from 0.9 to 40 keV[14,15]. Here we present details of the evidence for the 20 keV feature previously reported[16], but defer a complete discussion of the 12–180 keV spectral and temporal behaviour of the source. We caution that despite the attractiveness of the cyclotron interpretation of the feature, pending better understanding of the complex physics of radiation transfer in the magnetised, semi-relativistic atmosphere of an accreting neutron star, we cannot exclude the possibility that the observed phenomenon results from some quite different cause.

Observations

HEAO 1 pointed at 4U0115+63 (ref. 17) on 16 January 1978, when the source was near its peak brightness, for ≈3 h centred at ≈15.00 UT. Each of the two low energy detectors (LED1 and LED2) of the UCSD/MIT hard X-ray and low energy γ-ray experiment[18] had a ≈0.3 cm thick, actively shielded NaI 'phoswich' scintillation detector of area ≈100 cm² collimated to a 1.2° × 23° (FWHM) field-of-view by slat collimators with an energy resolution of ~6 keV (FWHM) at 20 keV. X-ray events in the 12–180 keV range are analysed in the 64-channel linear pulse height analyser (PHA) and transmitted event-by-event over a single telemetry channel shared among seven detectors. The mean residence time of events in the telemetry buffers determines the time resolution of about 0.05 s, binned here to 0.32 s. After elimination of Earth-blocked data and correction for electronic and telemetry dead times, each detector yielded about 6,000 s of useful data.

Fourier transforms and folds gave a best geocentric period of 3.61361 s during the observation, consistent with the SAS 3 ephemeris[12]. Figure 1 shows the data from LED2, folded modulo the pulse period and summed over the 12–66 keV interval in which we observe a significant pulsed flux. Comparison of this 'template' pulse shape with the data in individual PHA channels (Fig. 2) shows no strong variation of pulse profile with energy, but a marked reduction in pulse amplitude in the 19.3–24.8 keV band. In these circumstances it becomes possible to discuss the data in terms of a simple two-component model, in which we represent the observations as a sum of pulsed and steady fluxes. To determine the spectrum of each of these two components we fit the folded data in each PHA channel by the sum of two functions of the pulse phase—the first a constant, and the second given by the template. The smooth curves of Fig. 2 show the resulting fits. These smooth curves all have the same shape and phase alignment, which are those of the template. Only their amplitudes have been adjusted to fit the data. Because of minor differences in time and energy resolution and gain between the two detectors, we fit each independently, obtaining the spectra shown in Fig. 3.

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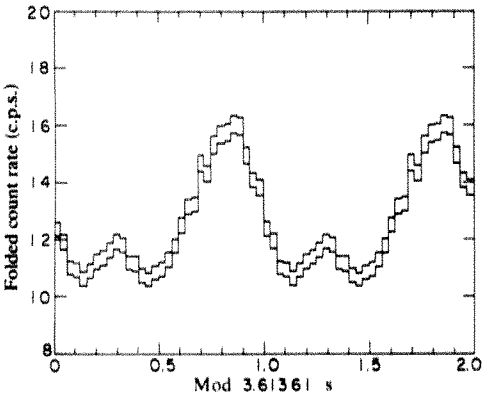


Fig. 1 Counting rates (12–66 keV) in low energy detector 2 (LED2) folded modulo the pulse period. LED1 shows a virtually identical profile. The upper and lower histograms show the data $\pm 1\sigma$, for two complete pulse periods. The data at any energy from 12 to 66 keV can be well represented by a sum of constant and a pulse of this shape. The plot includes a background count rate of 3.7 counts per second (c.p.s.). Phase 0 refers to JD 2443525.1134055.

The two lower sets of points (Fig. 3) show the pulsed spectrum in each detector, where we define the pulsed flux as the excess flux above the minimum of the fitted pulse profile, averaged over one 3.61 s cycle. Because the background is unpulsed, these spectra required no background subtraction, and their error bars reflect only counting statistics. This also allowed us to include data with the Earth's horizon in the field of view, which increases the useful exposure from the ≈3,800 s included in the total spectrum (and in our preliminary report[16]) to ≈6,000 s.

The large dip in the pulsed LED2 spectrum in PHA channels 9 and 10 corresponds to the low amplitude of pulsation noted in the 19.3–24.8 keV band of Fig. 2. The smaller dip in LED1 results partly from poorer resolution (since the feature is essentially unresolved—see below) in that detector, ≈33% (FWHM at 20 keV) as against ≈29% for LED2, and, we must conclude, partly from a statistical fluctuation. By a series of detector response calculations, detailed below, we have established that the combined probability (taking both detectors into account) that the observed feature could have arisen by a statistical fluctuation from a smooth continuum is very small ($\chi^2 = 50$ for $\nu = 7$ d.f.) but that adding a narrow absorption line to the model gives an improved χ^2 of 9.6 ($\nu = 5$) for the model of best fit. Because of the difference (in the depth the feature) between the two detectors, more complicated model photon spectra cannot be expected to improve χ^2 very much, so we accept the fit. For the model of best fit we obtained an absorption line energy $E_0 = 20.1 \pm 0.5$ keV and a line strength, expressed in terms of the equivalent width of continuum absorbed, of $\Delta E = 3.1 \pm 0.5$ keV. Independent fits to each detector separately yielded $\Delta E = 2.0 \pm 0.7$ keV for LED1 and $\Delta E = 4.1^{+1.1}_{-0.7}$ keV for LED2. Such an absorption line must have a depth exceeding 0.6 of the continuum level (95% confidence), which corresponds to a maximum actual width of about 5 keV (=3.1 keV/0.6). Systematic uncertainties, principally due to the idealised two-component analysis and to imperfections in the detector response models, may increase the absolute errors for E_0 and ΔE from the purely statistical errors quoted.

Experiments with different methods of analysis from those reported here, and with reasonable modifications of the detector response models, lead us to estimate the maximum likely increase in these errors to be less than a factor of two.

In these calculations we used response models for the two detectors based on pre-launch calibrations. We compared the expected counts for given input photon spectra with the actual data by a χ^2 test which treated the two detectors as a single experiment, summing χ^2 over the data from the five PHA channels nearest the feature in each detector. Systematic errors in the HEAO 1 attitude determination during the observation required independent normalisations for LED1 and LED2. Since we are concerned here only with the immediate vicinity of the feature, it was sufficient to use a simple form for the continuum, and we chose $dN/dE = A \exp(-E/E_\lambda)$. For the hypothesis of no feature we fitted for the continuum slope E_λ and the two normalisations, obtaining $\nu = 7$, and for the line hypothesis fits we added E_0 and ΔE, obtaining $\nu = 5$. We determined the single parameter, 1σ statistical errors in E_0 and ΔE, and the constraint on the line depth, using criteria discussed by Avni[19].

As the feature appears in the 3.61 s pulsed spectrum, it is very difficult to understand as an artefact of the instrument or analysis. It appears neither in the background nor in the unpulsed spectrum of 4U0115+63, nor in any other source spectra observed with the same instrument and analysed in the same way, whether pulsed (such as Her X-1, GX1+4, 4U0900−40 and 4U1626−67)[4,5,18] or total (Crab Nebula, Cyg X-1). We have divided the data into two halves, and the dip appears in each of them.

The two-component analysis which leads to the pulsed spectra of Fig. 3 has the important feature that, in the language of signal processing, it filters the data with the expected signal shape (the template), which yields optimal sensitivity. However, if there is a strong variation with energy of pulse profile or phasing this

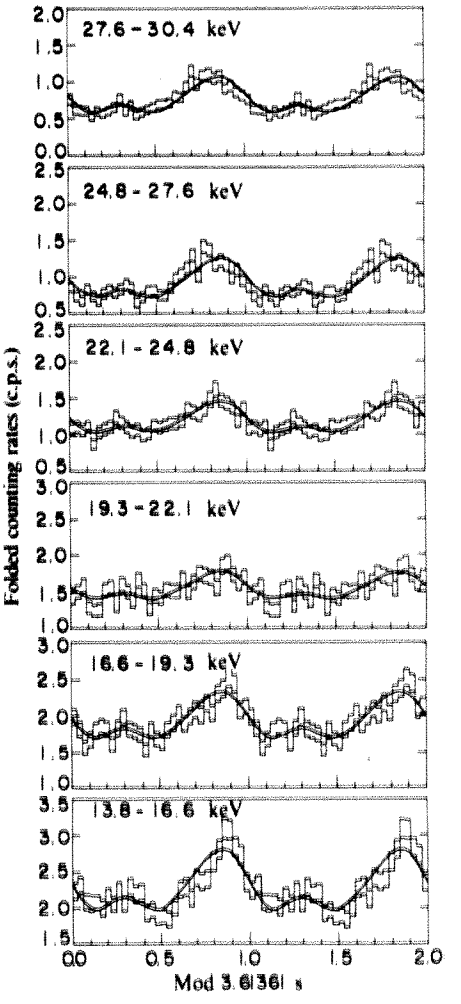


Fig. 2 Folded counting rates from LED2 in PHA channels 7–12, displayed similarly to Fig. 1, all to the same scale. The 19.3–24.8 keV channels (PHA 9 and 10) are noticeably less strongly pulsed than adjacent channels, possibly due to cyclotron resonance absorption. The continuous curves show fitted pulse profiles derived as described in the text, only smoothed for clarity by omission of all but the first three Fourier frequencies. Background rates for PHA channels 7 to 12 of 0.48, 0.37, 0.30, 0.26, 0.23 and 0.24 (c.p.s.), respectively, are included.

analysis could introduce spurious features. Data taken simultaneously by the Goddard Space Flight Center experiment on HEAO 1 show rapid changes in overall pulse shape with energy below about 10 keV (ref. 21) but examination of the data above 13 keV and the fits in Fig. 2 shows only small changes in pulse profile, which do not change the general shape or phase of the pulse, even in the channels with the dip, and do not account for the reduction in pulse amplitude there. A χ^2 test of the data in each PHA channel against the hypothesis of no pulsation gave $\chi^2 = 90$ (for 31 d.f.) in the channel with the dip, but $\chi^2 = 150$–190 at both higher and lower energies, which establishes the reality of the feature independent of any assumptions about pulse shape. Pravdo *et al.*[22] pointed out that incautious subtraction of physically different 'on-pulse' and 'off-pulse' spectra could produce an apparent absorption feature with no underlying physical significance. While one can never rigorously exclude such an explanation, the deep and narrow feature observed make it artificial in this case. We observe a pulse that varies strongly in amplitude but very little in shape as a function of energy. For such behaviour the picture of independent emission spectra with pulse phase offers no natural interpretation.

The two upper sets of data in Fig. 3 show the total (pulsed plus unpulsed) spectrum in each detector. In the analysis of these total spectra we used only data in which the Earth lay completely outside the collimator field of view in order to avoid large systematic background variations. We estimated the background from ≈4,000 s of sky exposure in each detector obtained in eight roughly equal intervals from the two orbits of scanning data adjacent to the observation; the errors shown for the total spectra include the effects of systematic variations in the background as estimated from the scatter among these eight intervals. Figure 4 shows the photon spectrum for LED2 corresponding to the total count spectrum of Fig. 3*b*, derived by dividing by detector efficiencies determined from the instrument response to the Crab Nebula. The spectrum is close to a power law with number spectral index ≈3.9 or to $\approx 10^8$ K thermal bremsstrahlung. A correction of 0.85 for the collimator transmission averaged over small aspect variations during the point was applied.

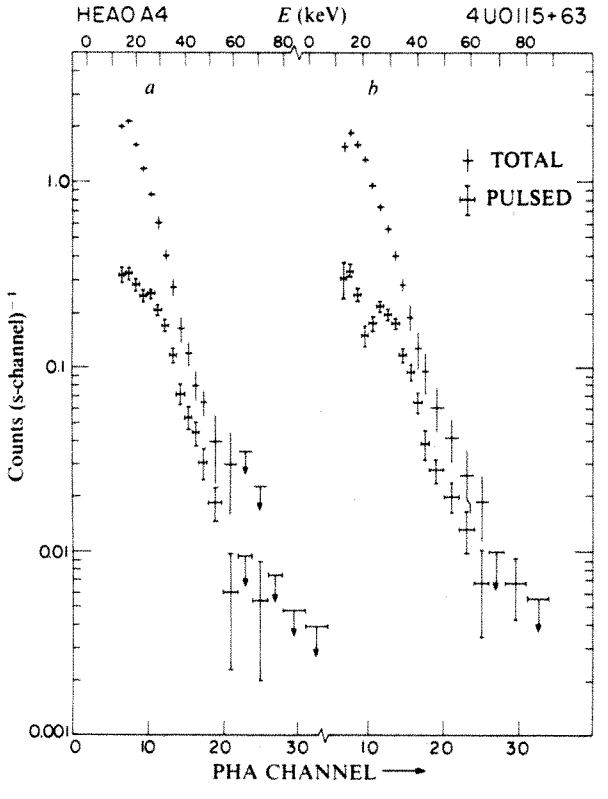


Fig. 3 Pulse height spectra of the total (upper) and pulsed (lower) counts in: *a*, LED1; and *b*, LED2. The 20 keV feature shows most clearly in LED2 in PHA channel 9. If it results from cyclotron resonance absorption, it corresponds to a surface magnetic field B of ~ 1.8–2.5×10^{12} G, (depending on the gravitational redshift), probably near the poles of the neutron star.

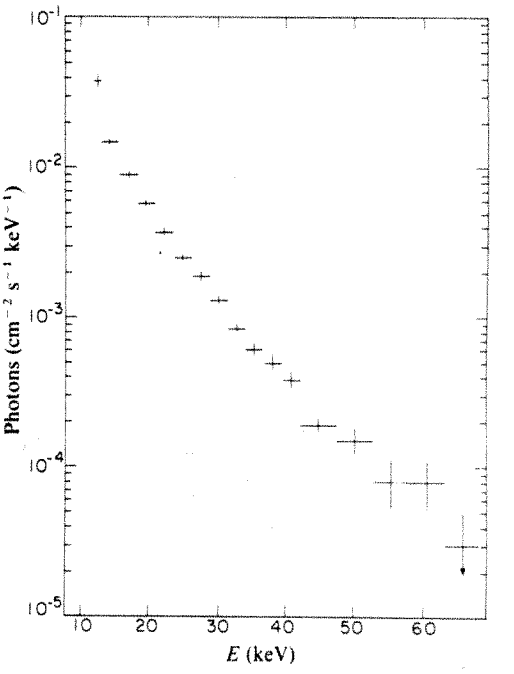


Fig. 4 Photon spectrum of the total flux (pulsed plus unpulsed), using the data from Fig. 3*b*, and based on a calibration using the Crab Nebula.

Discussion

Our results are consistent with all the other data known to us. However, the UCSD/MIT observation obtained about an order of magnitude more counts in the 20 keV range than any other instrument of comparable energy resolution. Hence no detailed confirmation of the 20 keV feature seems likely until further observations can be obtained.

Figure 3 shows that the pulse fraction, defined here as the ratio (pulsed flux)/(total flux), decreases from ≈50% at 60 keV to ≈17% at 15 keV. Johnston *et al.*[14] have found that it continues to decline to below 2.5 keV. Because the pulsed spectrum is harder than the total spectrum, the spectrum must harden near the peak of the pulse, behaviour generally similar to that seen from 0.9 to 18 keV by others[14,15]. However, the main peak observed below about 10 keV occurs at a different phase, about 0.5 instead of our 0.85.

Note that we use the term 'absorption' to include 'cyclotron resonance scattering'. The concavity of the total flux (Fig. 4) near 20 keV is sufficient to accomodate either absorption, which would entirely remove photons, or scattering, which could merely redistributate them in direction and thus pulse phase. However, as scattered photons could be beamed in directions never seen by Earth-bound observers, the two are essentially indistinguishable observationally.

The results for Her X-1 due to Trümper *et al.*[2] can be interpreted as absorption at energies below ≈42 keV as well as emission at ~58 keV. The data presented here, because of the deep and narrow feature, favour absorption/scattering as the correct interpretation for 4U0115+63 but cannot rule out an emission line explanation, in which the broad (about twice the instrument energy resolution) hump around 28 keV in the pulsed spectrum results from an emission blend seen over a steeply falling continuum. If due to cyclotron emission, our data would indicate a correspondingly higher and less uniform magnetic field. As we expect a cutoff in cyclotron emission corresponding to the maximum surface field near the magnetic

poles, the observed extension of the pulsed flux above the feature suggests an additional hard component of the continuum. No full theoretical treatment predicting the relative likelihood of cyclotron emission or absorption/scattering has yet appeared, though each has been briefly considered in the literature[3,23,24]. Dimensional estimates tend to support scattering/absorption over emission (F. Lamb, personal communication). Clear evidence for higher (or lower) harmonics could settle this question, but it does not appear in these data.

Assuming that the 20 keV feature arises near the surface of the neutron star, a gravitational redshift of ≤ 5–40% (ref. 25) gives 20–28 keV as the surface energy corresponding to a 20 keV line. If, following Trümper *et al.*[2] we interpret this as due to cyclotron absorption from the ground state ($j=0, s=-1$) to the first excited state ($j=1, s=-1$) or ($j=0, s=+1$) and assume negligible change in the electron momentum parallel to the magnetic field B, we obtain $B \approx (1.8 \text{ to } 2.5) \times 10^{12}$ G, probably near the base of the accretion column near the magnetic pole. The narrowness of the line implies it must arise in a region of fairly uniform field, since $\Delta B/B \approx \Delta E/E \leq 0.25$. Because the radial extent Δr of the absorbing region must satisfy $\Delta r/r \leq \frac{1}{3}(\Delta B/B)$ (ref. 2), if $r \approx 10$ km, then Δr must be <1 km. Since redistribution of photons in energy would tend to fill in a narrow absorption line, its width and strength limit the amount of reprocessing matter that can lie along the line of sight. Thus, this source does not seem to be observed through a cold magnetospheric shell of the type proposed for Her X-1 (refs 26, 27), as discussed in detail by Weaver[28].

As we have already constrained the height of the emission region to <1 km, any future improvements in resolution will begin to show whether it is more like the size of the accretion hot spot (≈ 1 km) or the atmospheric scale height (≈ 1 m). Thermal motions along the magnetic field line should also contribute to the linewidth. Since this effect depends on the cosine of the angle between the field and the line of sight, observation of the broadening as a function of pulse phase could eventually give both the temperature of the absorbing electrons and the geometry of the system.

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Plasma acceleration at the Earth's magnetopause: evidence for reconnection

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A characteristic of magnetic field reconnection is the acceleration of plasma as it flows across a rotational discontinuity. At the Earth's magnetopause this effect has only been observed recently during a few magnetopause crossings by the ISEE satellites. For one example analysed in detail the results show good agreement with theoretical predictions.

MAGNETIC reconnection or merging is thought to have a fundamental role in the conversion of energy stored in magnetic fields, and may occur in many cosmic situations. The concept of reconnection was introduced into magnetospheric physics by Dungey[1] who proposed that reconnection should be operative at the dayside boundary of the Earth's magnetic field, the magnetopause, as well as in the geomagnetic tail. A detailed description of the process was first provided by Petschek and coworkers[2,3] who demonstrated that reconnection may occur at substantial rates and that it leads to high-speed plasma jetting away from the reconnection site. Jets of plasma possibly associated with reconnection have been observed in the tail[4,5] but never at the magnetopause. This negative result has added to the controversy over the nature and efficiency of the reconnection process, despite substantial indirect evidence for its occurrence[6]. Recent measurements from the dual-satellite International Sun–Earth–Explorer Mission[7] (ISEE) have now shown the occasional presence of high-speed plasma at the magnetopause. We report here on one of these cases in which the observations agree in considerable detail with theoretical predictions.

The basic reconnection configuration for the magnetopause[3,8] is illustrated in Fig. 1. In this model, the magnetopause is a standing Alfvén wave emerging from the X-shaped centre of the

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reconnection site, referred to as the diffusion region. The magnetic fields on the two sides of the magnetopause current layer, which separates the magnetosheath and terrestrial fields, are not entirely tangential to that layer, but are connected and consequently have a small normal magnetic field component B_n. The magnetosheath plasma flows towards and across the magnetopause, or equivalently, there is a tangential electric field $\mathbf{E}_t$. As the plasma crosses the current sheet, its momentum changes abruptly under the action of the tangential components of the Maxwell stresses. Picturing the sharply bent field lines at the magnetopause as elastic bands acting on the plasma, the process may be likened to a magnetic slingshot, which ejects the plasma at high speed in poleward-directed jets just inside the magnetopause. The energy added to the plasma in this manner is extracted from the electromagnetic field[9] at the rate $\mathbf{E}_t \cdot \mathbf{I}$, where $\mathbf{I}$ is the total magnetopause current.

Observations

Figure 2 shows plasma and magnetic field observations which demonstrate the behaviour described above. The plasma data were obtained with the LASL/MPE quadrispherical analysers[10], which measure the three-dimensional distribution function of ions and electrons with very good time resolution. Full distributions are obtained in three spacecraft revolutions, or

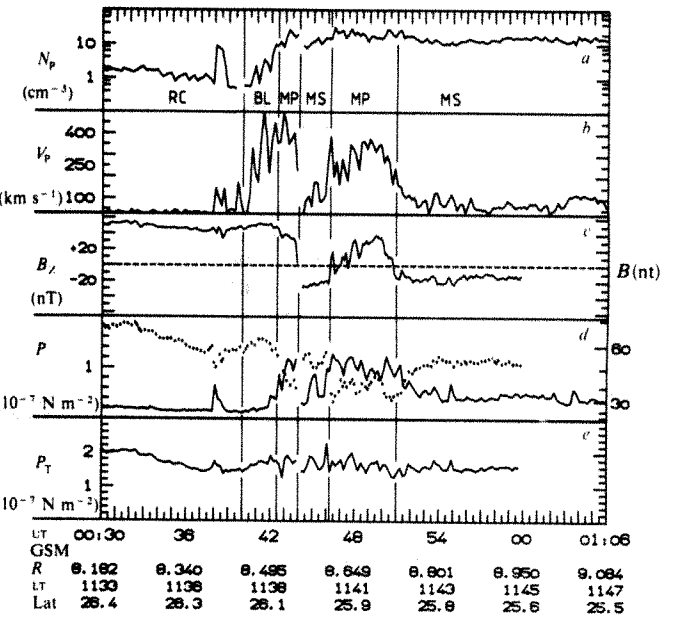
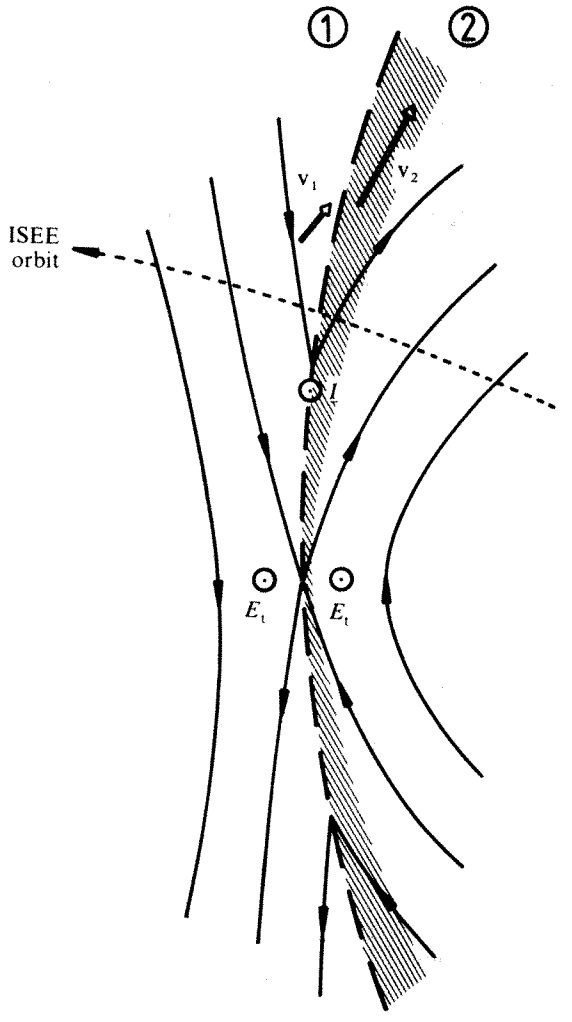


Fig. 2 Plasma and magnetic field data for the ISEE 1 magnetopause crossings on 8 September 1978. *a*, *b* and *c* show the proton density N_p, the proton flow speed v_p, and the magnetic field component B_z, perpendicular to the ecliptic. In *d* the solid line indicates the plasma pressure P, defined as $N_p k(T_p + T_e)$, where k is the Boltzmann constant and T_p and T_e are the proton and electron temperatures, respectively. The dotted line represents the magnetic field strength B, when using the scale on the right, or the magnetic field pressure $B^2/2\mu_0$ with the scale on the left. *e*, The total pressure $P + B^2/2\mu_0$. Plasma parameters are spaced 12 s apart, magnetic field quantities 8 s apart. During the 36 min shown, ISEE 1 moves from a radial distance, R, of 8.2 to 9.1 Earth radii. The local time, LT, is slightly pre-noon throughout the period, the latitude, Lat, stays near 26° (all coordinates in the geocentric solar magnetospheric, GSM, system). Initially ISEE 1 is located in the outer magnetosphere or ring current (RC) region. It then traverses a thin boundary layer (BL) of magnetosheath-like plasma. Due to boundary motions there are multiple magnetopause (MP) encounters before the satellite exits into the magnetosheath (MS). The magnetopause encounters are identified by the transitions in B_z from northward (positive) in the magnetosphere to southward in the magnetosheath.

Fig. 1 Reconnection configuration for southward-directed external magnetic field. Solid lines denote magnetic field lines. The dashed line marks the magnetopause, and the shaded area the boundary layer. Magnetosheath and magnetosphere are located to the left and right of the magnetopause, respectively. The normal magnetic field component B_n is negative at the ISEE orbit.



~9 s, and repeated every 12 s. This time resolution which is absolutely necessary in magnetopause studies, is achieved at the expense of energy/angle resolution. For ions, measurements are made over an energy range between 70 eV and 40 keV per charge in 16 channels, covering elevation angles between +55° and −55° in four segments, and 360° of azimuth in eight and four equidistant sectors for the inner and outer elevation segments, respectively. The plasma data are shown as moments of the measured distribution functions[11]. Data from the UCLA triaxial fluxgate magnetometer[12] are presented here as 12-s averages overlapped by two-thirds so that there is one sample every 4 s.

The data shown in Fig. 2 are from an outbound traversal of the subsolar magnetopause region at a latitude of ~25°. If we assume that the diffusion region of the reconnection configuration was located near the subsolar point, the measurements were made along a path indicated in Fig. 1. Magnetopause (MP) encounters are identified by the changes in field orientation (B_z panel). Due to motions of the magnetopause, the first, complete crossing is followed by another, incomplete one, before the satellite finally exits into the magnetosheath (MS).

A magnetopause speed of 10 km^{-1} s relative to the spacecraft is derived for the first crossing from the time difference between the ISEE 1 and ISEE 2 crossings, the latter spacecraft being 1,820 km further away from the Earth. Using 10 km s^{-1}, a magnetopause thickness of 900 km is deduced, which is large compared with an ion gyro radius (~100 km). Figure 2*a* shows that the plasma number density, N_p, was within a factor of 1.5 of the average magnetosheath value during the magnetopause encounters. However, earthwards of the inner edge of the first

crossing, the density dropped gradually to the low values of the outer magnetosphere. This period, denoted BL, corresponds to the low-latitude plasma boundary layer[13,22]. The density peak within the magnetosphere presumably represents an early partial entry into the boundary layer.

The plasma pressure P and the magnetic field magnitude B show substantial variations during the magnetopause crossings. However, as Fig. 2*e* shows, the total pressure $P_T = P + B^2/2\mu_0$ remained approximately constant as expected.

The most significant feature shown in Fig. 2 is the extremely large plasma flow speed, V_p, observed during each magnetopause encounter and persisting throughout most of the boundary layer. These high speeds, which were also seen by ISEE 2, peak at 450 km s^{-1}, whereas values in the adjacent magnetosheath fall in the range 50–100 km s^{-1}. The latter speeds are consistent with the prediction of gas-dynamic models for this location[14]. As already mentioned, enhanced flow speeds at the magnetopause are characteristic of reconnection.

Comparison with theoretical expectations

We now compare the observations quantitatively with the predictions of the reconnection model of the dayside magnetopause[3,8]. In this model the magnetopause is described as a rotational discontinuity—a current layer which possesses a normal magnetic field component and across which the tangential field changes direction by an arbitrary angle. For such a sheet the following relations hold[15]

$$\mathbf{v}_{t2} - \mathbf{v}_{t1} = \pm((1-\alpha_1)/\mu_0\rho_1)^{1/2}[(\rho_1/\rho_2)\mathbf{B}_{t2} - \mathbf{B}_{t1}] \quad (1)$$

$$\rho_2/\rho_1 = (1-\alpha_1)/(1-\alpha_2) \quad (2)$$

$$v_n = \pm B_n((1-\alpha)/\mu_0\rho)^{1/2} \quad (3)$$

$$\alpha \equiv (P_\parallel - P_\perp)\mu_0/B^2 \quad (4)$$

Here **B**, **v** and ρ are the magnetic field vector, plasma flow velocity and mass density, respectively, and the subscript pairs (n, t) and (1, 2) refer to components normal (n) or tangential (t) and exterior (1) or interior (2) to the magnetopause surface; $P_\parallel$ and $P_\perp$ are the pressures parallel and perpendicular to the magnetic field, respectively, and α is the pressure anisotropy factor. Equation (3) shows that the plasma flows across the sheet with the modified Alfvén speed. Thus, a rotational discontinuity is in fact a large amplitude Alfvén wave. Density changes across such a wave occur only when the anisotropy factor changes, as indicated by equation (2). Finally, equation (1), multiplied by ρv_n, expresses the balance of tangential stresses in the current sheet. The left-hand side is the rate of change of tangential momentum of the plasma as it flows across the layer while the right-hand side, using equations (2) and (3), is seen to represent the sum of the net tangential Maxwell stress $\tau_M = (B_n/\mu_0)(\mathbf{B}_{t2} - \mathbf{B}_{t1})$ and pressure anisotropy stress $-\alpha\tau_M$. Assuming $v_n < 0$ (see Fig. 1), the positive and negative signs in equations (3) and (1) refer to $B_n < 0$ and $B_n > 0$, respectively.

Equation (1) forms the basis for our test of the hypothesis that the plasma inside the magnetopause received its high velocity from the magnetic slingshot. We apply it across the first magnetopause crossing, using only data points immediately adjacent to the current sheet. The number density, although it had a substantial maximum within the sheet, had nearly identical values (~10 cm^{-3}) on the two sides. Equation (2) then indicates that α also should have been the same. For $\rho_2 = \rho_1$ equation (1) predicts that the tangential velocity difference vector should be parallel ($B_n < 0$) or antiparallel ($B_n > 0$) to the magnetic difference vector, and that its magnitude should equal the modified Alfvén speed based on the magnetic difference field. Figure 3 shows the behaviour of **v** and **B** throughout the first magnetopause crossing. The two difference vectors may be imagined as extending from points ① to points ② on the left in Fig. 3. They are seen to be nearly parallel, forming an angle of

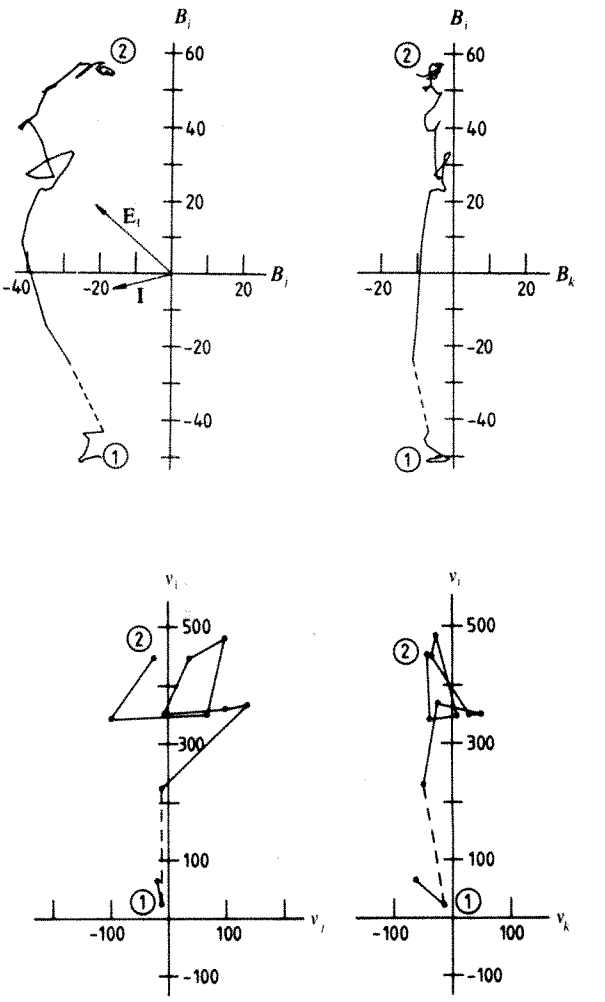


Fig. 3 Polar plots of the magnetic field **B** (nT) and plasma flow velocity **v** (km s^{-1}) for the first magnetopause crossing of Fig. 2 in the principal axes coordinate system obtained from minimum variance analysis. The k axis is in the direction of minimum variance of the magnetic field and is interpreted as the outward-directed normal to the magnetopause. The (i, j)-plane is tangential to the magnetopause, with the i and j axes approximately due north and west, respectively. Direction cosines with respect to the solar ecliptic x, y, z axes are (−0.59; −0.37; 0.72), (−0.12; −0.84; −0.53) and (0.80; −0.40; 0.45) for the i, j, k axes, respectively. The points marked 1 and 2 refer to the magnetosheath and magnetosphere sides of the magnetopause. Magnetic field and plasma data points are separated by 4 and 12 s, respectively. Data gaps are shown as dashed line segments. Also shown are vectors representing the magnetopause current **I**, and the electric field $\mathbf{E}_t$.

only 12°. Given the uncertainties associated with temporal variations and with the plasma measurements, this value is not significantly different from zero. The fact that the vectors are parallel rather than antiparallel indicates $B_n < 0$, as expected for a crossing in the Northern Hemisphere (see Fig. 1).

The magnitude of the measured tangential velocity difference is 427 km s^{-1} whereas for $\alpha = 0$, a number density of 10 cm^{-3}, and a 5% abundance of α particles in the proton plasma, the Alfvén speed based on the difference magnetic field is 580 km s^{-1}. We conclude that the difference between these two numbers is not significant. First, gain degradation of the plasma detectors may have led to a systematic underestimation of the density of up to 20% and therefore to an overestimation of the difference Alfvén speed of up to 10%. More importantly, the limited angular resolution of the measurements will lead to an uncertainty (normally an underestimate) of the flow velocity of up to 30% if the flow is inclined ~45° with respect to the ecliptic, as in the present case[11]. Finally, the presence in the magnetopause region of α particles beyond the assumed level and/or of small numbers of heavier ions of ionospheric origin may reduce the difference Alfvén speed further.

Figure 2 shows that high velocity plasma is present, not only in the boundary layer but throughout most of the magnetopause itself, both for the main crossing and for the partial ones. As the magnetopause thickness was large compared with an ion gyro radius, we may compare all of the velocity measurements with equation (1) as follows: We suppress the subscript 2 in that equation and let the corresponding quantities represent conditions at an arbitrary point. Subscript 1 will represent average magnetosheath conditions. Equation (1) then predicts a linear relationship, with slope

$$m = [(1-\alpha_1)/\mu_0\rho_1]^{1/2} \quad (5)$$

between $(\rho_1/\rho)\,\mathbf{B}_t$ and $\mathbf{v}_t$. As Fig. 3 shows, the main dynamical effect is associated with the 'i' components of these two vectors. A scatter plot of $\mathbf{v}_i$ against $(\rho_1/\rho)\,\mathbf{B}_i$ is shown in Fig. 4. Linear regression analysis of these data yields a correlation coefficient of 0.90 and a slope of 4.4 ± 0.3 (km s^{-1} nT^{-1}). On the other hand, the slope obtained from equation (5) with $\alpha_1 = 0$ and an average magnetosheath particle density ρ_1 (see Fig. 2) of 12.8 cm^{-3}, is $m = 5.6$ (km s^{-1} nT^{-1}). In view of the uncertainties discussed previously, the difference between the two slopes is not significant. We conclude that the entire set of data is compatible with the slingshot acceleration hypothesis.

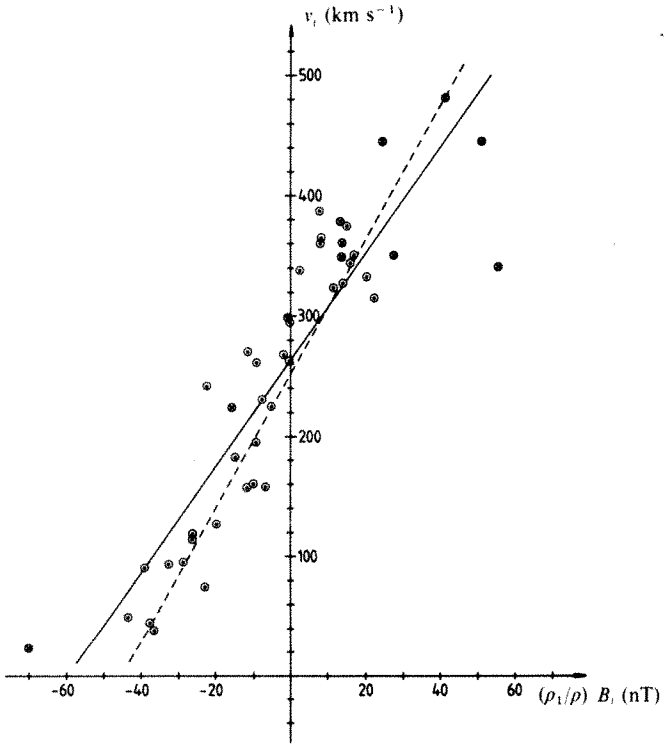


Fig. 4 Scatterplot of v_i against $(\rho_1/\rho)\,B_i$ for the magnetopause encounters of Fig. 2. ⊗, 00.42.20–00.44.20 UT interval; ⊙, 00.45.56–00.52.56 UT interval. v_i and B_i are the components of $\mathbf{v}_t$ and $\mathbf{B}_t$ along the i axis in Fig. 3. The index 1 refers to average magnetosheath conditions ($\rho_1 = 12.8$ cm^{-3}). Index-less symbols refer to measurements in the intervals above. The solid line is a fit to the data using a linear regression analysis, the dashed line marks the slope predicted by equation (5) with $\alpha_1 = 0$ and $\rho_1 = 12.8$ cm^{-3}.

As already mentioned, the slingshot acceleration process requires the presence of a magnetic field component B_n perpendicular to the magnetopause. Furthermore, the direction change of the tangential plasma velocity in the event discussed here corresponds to a negative value of B_n. To check this prediction, we have calculated the normal vector $\mathbf{n}$ and the average normal magnetic field component $\mathbf{B}_n$ directly from the magnetic data during the first crossing by the minimum variance technique[16,17]. The results depend on the precise time interval chosen, with B_n ranging from −3 to −9 nT. The normal coordinate system used in Fig 3, and the subsquent calculations of v_n and E_t are based on the results from a time segment with $B_n = (-5.4 \pm 2.9)$ nT. This value is close to the average and has the smallest absolute error. With $B_n = -5.4$ nT, an average density $N_p = 15$ cm^{-3} and $\alpha = 0$, we find (equation (1)) $v_n = -28$ km s^{-1} for the first magnetopause crossing. Because of the inward motion, at 10 km s^{-1}, of the magnetopause relative to the satellite, the predicted normal plasma velocity is −38 km s^{-1}. As shown in Fig. 3, the measured velocity component v_k is in fact negative on the average, albeit with large fluctuations. Figure 3 illustrates the difficulty of establishing the presence or absence of plasma flow across the magnetopause by direct examination of v_k.

In a time-dependent one-dimensional magnetopause, the tangential electric field $\mathbf{E}_t$, measured in the frame moving with the magnetopause, should remain constant during a crossing. This field may be obtained from the simplified Ohm's law[18]:

$$\mathbf{E}_t = -\mathbf{v}_n \times \mathbf{B}_t - \mathbf{v}_t \times \mathbf{B}_n \quad (6)$$

The largest correction term to equation (6) would be the Hall term[18]. However, for a thick layer this term contributes very little. Using the values of B_n and v_n derived previously, we find $E_t = 1.8 \pm 0.5$ mV m^{-1} during the first crossing. The electric field vector forms an angle of $56° \pm 10°$ with the total current $\mathbf{I}$, which was deduced from the jump in $\mathbf{B}_t$ (see Fig. 3). This result supports Cowley's[19] conclusion, arrived at from theoretical considerations, that the two vectors need not be parallel during reconnection.

The dissipation rate $\mathbf{E} \cdot \mathbf{I}$, with $I = 0.073$ A m^{-1}, is found to be 78 ± 24 W km^{-2}. Both the electric field and the dissipation rate are comparable to those obtained from the d.c. electric field experiment[20] on ISEE 1 for another crossing[21]. Unfortunately, that experiment was in an unfavourable mode during the present event, so that no comparison is possible.

Conclusions

Our observations provide the first evidence for the occurrence of the plasma acceleration at the magnetopause intrinsic to the reconnection process. So far two more examples of this kind have been found in the ISEE data. However, in most cases with favourable magnetic field orientation no plasma acceleration is observed. This means either that reconnection occurs rarely and in conditions which are not yet understood, or that the process is small-scale and non-stationary, and therefore has a low probability of being observed locally.

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Rare earth element mobility associated with uranium mineralisation

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Extreme rare earth element mobility has been discovered, associated with uranium mineralisation in metasedimentary rocks of the Lower Proterozoic Pine Creek Geosyncline, Australia. The rare earth element patterns and other geochemical evidence point to movement of the rare earth elements and uranium by carbonate complexes in a low temperature, oxidising and alkaline solution.

MOBILITY of rare earth elements (REEs) in rocks is of interest as these elements are often used as petrogenetic indicators. The REEs seem to be immobile in most metamorphic conditions[1-3]. Some light rare earth element (LREE, La–Sm) mobility has been detected during hydrothermal alteration (submarine weathering), spilitisation and lower greenschist metamorphism associated with hydrothermal activity[4-6]. Collerson and Fryer[7] suggested heavy rare earth element (HREE, Gd–Lu) mobility due to carbonate complexing associated with granulite metamorphism and granite production in Archean terrains. Several studies have related HREE mobility to complexing (mainly carbonate complexing) in hydrothermal conditions[8-13]. The present study examines the relationship between REE and uranium mineralisation in the Cahill Formation[14] of the Pine Creek Geosyncline, Northern Territory, Australia.

Geological setting

The following summary of the geology and geochemistry of the Pine Creek Geosyncline is based on information given at the International Uranium Symposium on the Pine Creek Geosyncline, Sydney, 3–8 June, 1979.

The Pine Creek Geosyncline (Fig. 1) comprises up to 14 km of mainly Lower Proterozoic metasedimentary rocks (clastics and carbonates)[15]. These rocks were deposited between age limits of 2,400 and 1,870 Myr (ref. 16).

Most of the important uranium deposits in the eastern part of the Pine Greek Geosyncline are contained within the Cahill Formation (Fig. 1). This unit consists primarily of quartzo-feldspathic and micaceous metasedimentary rocks. A lower member (which contains most uranium-deposits) contains abundant carbonate and carbonaceous metasedimentary rocks[15]. The exact age of the Cahill Formation is in some doubt. Most workers[14,15,17] suggest it is of Lower Proterozoic age. Consideration of metamorphic assemblages[18], however, indicates that these rocks may have been buried deeper than can be explained by the known Lower Proterozoic sedimentary thickness and that an age as old as Archean cannot be excluded.

Uranium mineralisation, for the most part, is strata-bound and occurs in breccia matrix and vugs. In most samples, chlorite is ubiquitous, primarily as an alteration product[19]. The favoured model is that uranium deposition occurred from a low temperature fluid within collapse structures (caves, cavities) of an impure carbonate stratum which has undergone extensive solution and development of karst terrain[20]. Alternative models have been described by Binns *et al.*[21] and Crick and Muir[22]. Ore deposition probably occurred between about 1,800 and 1,700 Myr (refs 20, 23).

Most samples analysed in this study probably represent the insoluble residue (plus alteration products, ore minerals and so on) after carbonate had been removed. Samples include fine-grained metasedimentary schists with a wide range of uranium concentrations (1.4–48,230 p.p.m.). The least altered sample (PCG132) is a muscovite–biotite–quartz schist (SiO_2 = 77.44%). Modal mineralogy of the altered samples is rather variable although chlorite (primarily as an alteration product) and quartz are always dominant. Muscovite, graphite and haematite are also occasionally present, in most samples. One sample of massive, impure uraninite (uraninite + fourmarierite + secondary U and Pb minerals) was also analysed.

REE, Th and U data

Four samples were analysed for REE (excluding Pr + Ho) by an ion-exchange/X-ray fluorescence technique and the remainder by spark source mass spectrometry. Details of techniques have been reported previously[24,25]. Uranium and thorium concentrations on samples PCG52, PCG57 and PCG148 and U concentration on sample 75080180 were made available by John Ferguson of the Bureau of Mineral Resources, Canberra, Australia. Results of REE, U and Th analyses are given in Table 1.

As most of these rocks were originally sedimentary, it is convenient to compare REE data to typical sedimentary rocks. Figures 2 and 3 plot the REE data normalised to Post-Archean Average Australian Sedimentary rock (PAAS)[26].

Sample PCG132 has a U concentration (1.4 p.p.m.) and Th/U ratio (4.7) well within the range of typical sedimentary rocks and may be considered a normal, unmineralised sample. The REE pattern of this sample (Fig. 2) is nearly parallel to PAAS, although lower in total abundances by a factor of about 0.5–0.7. Minor HREE depletion is apparent and there is no anomalous Eu behaviour relative to PAAS. The normal distribution of REE in clastic sedimentary rocks is extremely uniform and similar to PAAS[26]. It is therefore likely that PCG132 is fairly typical of unaltered samples in the Cahill Formation.

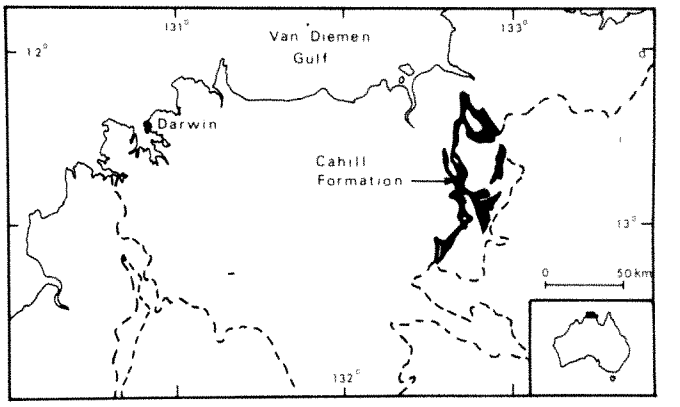


Fig. 1 Location map of the Pine Creek Geosyncline, N.T., Australia. The approximate limits of the geosyncline are marked by dashed lines and the Cahill Formation is shown in black.

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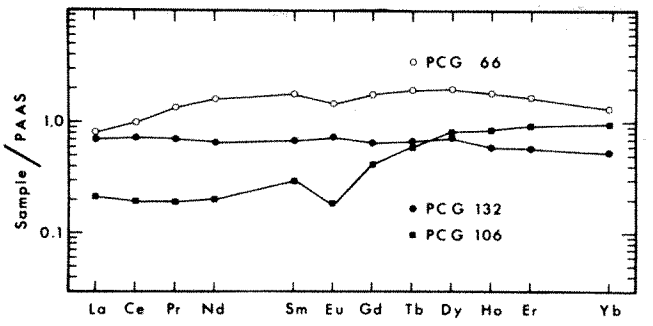


Fig. 2 REE patterns, normalised to post-Archean average Australian shales (PAAS)[26,36], for samples with low and intermediate U content.

Samples with intermediate U content (PCG66, PCG106) show REE patterns (Fig. 2) which differ substantially from PAAS but there is no consistent trend. PCG106 is notable for LREE depletion and a negative Eu anomaly. Samples enriched in U(PCG57, PCG148, PCG52, 75080180) show consistent REE trends (Fig. 3). They are depleted in LREE and enriched in HREE. These samples show a PAAS-normalised maximum in the vicinity of Tb–Dy.

The impure uraninite sample (75080260) has a REE pattern which parallels the U-rich samples (Fig. 3). Uraninite alone, however, cannot account for the REE concentrations of the other samples. Mixing calculations between PCG132 and 75080260 result in about an order of magnitude excess in total REE (ΣREE) for most samples with U concentrations comparable to those resulting from the mixing proportions. Also, LREE depletion cannot be achieved by such mixing.

For all metasedimentary rock samples, there is an inverse correlation between U content and La:Yb ratio (Fig. 4). Samples with U ≥ 1,900 p.p.m. show a correlation between U content and ΣREE (see Fig. 3). Another interesting feature of these rocks is the complete lack of coherence between U and Th, as indicated by the strong inverse correlation between U content and Th:U ratio (Fig. 5). This is in strong contrast to the general behaviour of Th and U in most igneous and sedimentary rocks[27].

The remarkable correlation between REE and U content, in these samples, has not been previously observed. The REE patterns in the U-rich samples (Fig. 3) are unlike any terrestrial[3] or extraterrestrial[28] rock. Common rock forming minerals such as garnet and zircon do display HREE enrichment[3], but they are not present in significant proportions and could not explain these REE patterns. No REE minerals have been identified in these samples which could explain such patterns and it is possible that much of the REE content was associated with the original clay minerals and alteration products (now chlorite, biotite, muscovite, and so on). Significant adsorption of REE on clay minerals has been documented for Quaternary clays of Norway[29].

REE mobility

The above observations indicate that massive REE mobility related to U movement, resulting in depletion of LREE and enrichment of HREE, occurs relative to the parent rock. The

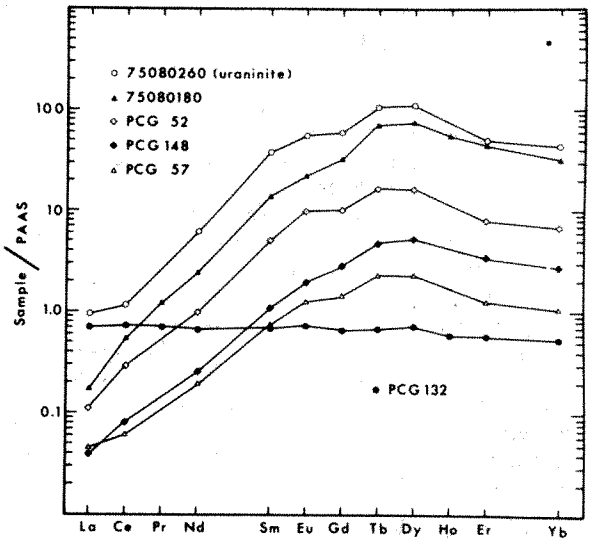


Fig. 3 REE patterns, normalised to PAAS, for samples with high U content. Sample PCG132 shown for comparison.

Table 1 Trace element analyses of samples from the Cahill Formation, Pine Creek Geosyncline (p.p.m.)

Sample	PCG52	PCG57	PCG66	75080180	PCG106	PCG132	PCG148	75080260	PAAS§
Th	16	13	20	15	18	6.6	9	—	
U	12,580	1,900	455	48,230	229	1.4	3,720	—	
Th/U	0.0013	0.0068	0.044	0.0003	0.079	4.7	0.0024	—	
La	4.2	1.7	31	5.5	7.9	27	1.5	30	38
Ce	23	4.8	79	43	15	58	6.5	92	80
Pr	—	—	12	11	1.7	6.2	—	—	8.9
Nd	32	6.2	52	77	6.5	21	7.9	200	32
Sm	28	4.2	10	79	1.7	3.9	5.8	214	5.6
Eu	11	1.4	1.6	24	0.20	0.80	2.1	61	1.1
Gd	47	6.6	8.4	153	2.0	3.1	13	277	4.7
Tb	13	1.8	1.5	55	0.47	0.53	3.6	83	0.77
Dy	73	10	8.8	329	3.6	3.2	23	490	4.4
Ho	—	—	1.8	55	0.84	0.58	—	—	1.0
Er	23	3.6	4.8	128	2.7	1.7	10	144	2.9
Yb	19	2.9	3.7	89	2.6	1.5	7.9	127	2.8
ΣREE†	294.8	47.2	215.8	1075.5	46.0	128.0	89.3	1853	
Eu/Eu*‡	0.94	0.82	0.54	0.68	0.34	0.71	0.75	0.78	
La/Yb	0.22	0.59	8.4	0.062	3.0	18.0	0.19	0.24	

Samples used—PCG52 (graphite–chlorite–quartz schist); PCG57 (chlorite–quartz schist); PCG66 (chlorite–muscovite–quartz schist); 75080180 (quartz–mica–chlorite–haematite schist); PCG106 (chlorite–muscovite–quartz schist); PCG132 (muscovite–biotite–quartz schist); PCG148 (muscovite–chlorite–quartz schist); 75080260 (impure uraninite) and PAAS.

† Estimated.

‡ Eu* is a theoretical value for no chondrite-normalised Eu-anomaly.

§ PAAS—Post-Archean average Australian sedimentary rock; values from Nance and Taylor[26].

LREE depletion shows that REE were leached from the sedimentary rocks themselves. The HREE were selectively deposited with the U or selectively incorporated into the ore depositing solution. A HREE-depleted parent lithology has not been identified but slight HREE depletion was apparent in PCG132, the supposedly unaffected sample. If large volumes of parent rock were involved, a notable HREE depletion may not occur.

The nature of the solution which carried the REE and U can be determined from several lines of evidence. The lack of Th–U coherence (Fig. 5) strongly suggests that U was primarily transported in solution as U^{6+}. Complexes of uranous ions with chlorides, sulphates, phosphates or fluorides are thus unlikely transporting mechanisms[27,30]. Transport of U as uranyl ions (UO_2^{2+}), carbonate complexes, uranyl fluoride complexes or uranyl phosphate complexes are all possible[30]. Although several secondary uranium sulphate and uranium phosphate minerals have been reported from this area, lack of correlation between U and S, F or P^{32} suggests that sulphate, fluoride and phosphate complexes were unimportant.

REE mobility, under hydrothermal conditions, is also best accomplished by soluble complexes. The most common forms of complexing are sulphate, fluoride and carbonate[8]. Sulphate complexing generally results in major hydrothermal sulphate deposits[8]. Pseudomorphs after sulphate minerals have been recognised in this region but are thought to be of evaporitic origin[22]. The lack of correlation between REE (La, Ce, Y) and F or S^{32} indicates that these complexes were probably unimportant.

It seems most likely that U and REE were transported together in a hydrothermal fluid as soluble carbonate complexes. Typical forms would be $[UO_2(CO_3)_3]^{4-}$ and $[REE(CO_3)_3]^{3-}$. Transport of U as simple uranyl ions may also have occurred but is not considered important since this species only predominates at relatively low *p*H[30]. The shape of the REE patterns for the U-rich samples is easily explained by such a mechanism since complexes of the HREE tend to be more soluble than LREE[8].

Strong supporting evidence for this mechanism is available from fluid inclusion studies[33]. Inclusions in quartz, thought to be associated with the main stage of U-mineralisation (Type II inclusions of Ypma and Fuzikawa[33]) contain CO_2 as a major phase (along with H_2O and CH_4). The temperatures of such fluids are difficult to determine but very low (meteoric?) values have been suggested[20]. REE and U carbonate complexes of the type suggested here would be best formed under alkaline ($pH \geq 7.5$) and oxidising ($E_H \geq 0.2$ V) conditions with temperatures below about 100 °C (refs 8, 30).

The conditions in which deposition occurred are more speculative. Lowering of *p*H, E_H and carbonate activity are all efficient mechanisms[8]. The activity of CO_2 would be effectively

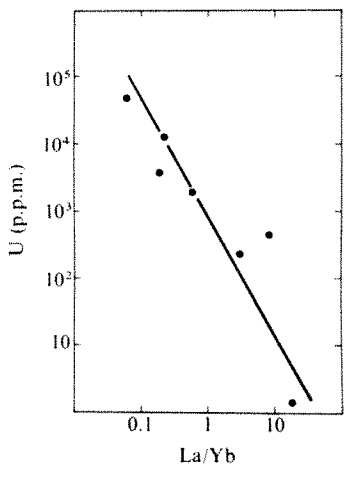


Fig. 4 Plot of U content against La/Yb for whole rock samples.

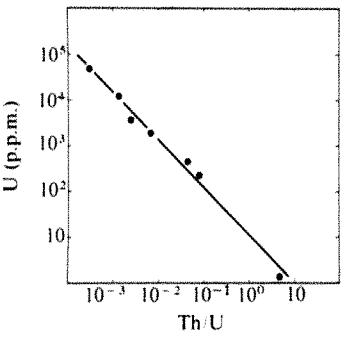


Fig. 5 Plot of U content against Th/U for whole rock samples.

decreased by the lowering of total pressure on entrance of the ore-bearing solutions into cavities[8]. Such a mechanism would be in accordance with the karst environment suggested for these ores[18,19].

Most other studies which have appealed to carbonate complexes for mobilisation of REE have dealt with HREE depletion[7,13]. These studies, however considered fairly high temperature regimes. In a study of the Precambrian Sokoman iron-formation, Fryer[12] suggested REE mobility by carbonate complexes during diagenesis to explain HREE enrichment. It seems that HREE are preferentially retained in hydrothermal solutions and only removed in late stage and/or low temperature deposits. This is consistent with present understanding of chemistry of REE carbonate complexes[8,9].

A final point which deserves comment is the behaviour of Eu in these rocks. If soluble carbonate complexes are controlling REE distribution in these rocks, the Eu^{2+} would be expected to behave very differently than REE^{3+}. This should result in anomalous Eu behaviour with increasing U-content; this clearly does not occur (Fig. 3). It seems unlikely that a second major complexing agent was present (such as halogens and phosphates) allowing transport of Eu^{2+} (see above discussion). Equally unlikely is the possibility that Eu^{2+} was oxidised to Eu^{3+} by the ore-forming fluid since other evidence[20] suggests that the fluid varied only from moderately reducing to moderately oxidising. An intriguing possibility is that the source material contained only Eu^{3+}. If the source were sedimentary it may indicate that Eu^{2+} can be oxidised to Eu^{3+} during sedimentary processes and still preserve a memory of Eu-anomalies inherited from the igneous precursors[26,34–36].

These results have important implications for a wide range of geochemical problems. It is now quite clear that the REEs can be transported as soluble carbonate complexes in fluid phases. REE studies on rocks which have been significantly affected by fluid migration and alteration must take this factor into account.

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Vesicularity and CO_2 in mid-ocean ridge basalt

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Vesicles and included CO_2 are enriched in deep-sea basalts that are also enriched in light rare earth and incompatible elements. This enrichment probably results from a unique deep mantle origin of such melts but may have been modified by CO_2 bubbles rising in shallow magma chambers.

THE primary control on the vesicularity of subaqueously erupted basalt is the depth (and hydrostatic pressure) of eruption[1,2]. Shallower eruption produces more (and larger) vesicles not only because exsolved volatiles expand with lower pressure, but also because exsolution of volatiles dissolved in the melt is more complete. However, basalt composition also affects the vesicularity of submarine basalt. Alkalic basalt seems to be more vesicular (at the same depth) than tholeiitic basalt[3], although fresh submarine alkalic basalt sample suites are rare and data limited. Fresh tholeiitic basalt from the same general area on a mid-ocean ridge such as the Mid-Atlantic Ridge at 37 °N shows a variation in vesicularity that can be related to major element variations, such as K_2O (ref. 4).

In addition to these vesicularity controls, important variations have been noted between tholeiitic basalts from different mid-ocean ridges which seem to be independent of depth or major element chemistry[5]. These variations are of such magnitude that individual suites from mid-ocean ridges can be characterised by their vesicularity.

Analyses of the gas contained in vesicles from four localities on spreading ridges indicates that >95% of the remaining gas is CO_2 (ref. 6). Hence the vesicularity of submarine basalts (except for those that are shallower than about 1 km) is a measure of the CO_2 content of the lava (other than that dissolved in the glass).

In this study, vesicularity has been determined by point counting standard thin sections of the outermost 1–1.5 cm of pillows and slabs, most of which include at least some sideromelane glass. More than 1,000 points were counted, and the vesicularity is reported in volume per cent of vesicles in glass (or microcrystalline material) excluding phenocrysts and microphenocrysts. More than 200 samples have been analysed from 13 ridge segments in the Atlantic and Pacific oceans (Table 1).

Results

The measurements of the volume per cent vesicles at any locality vary widely, particularly in rocks with a low vesicle content, but show a rough log-normal distribution (Fig. 1). For lava suites with ~10% vesicles, the variation is about one-half order of magnitude, but for suites of low vesicularity, the variation is as much as 1.5 orders (Fig. 1). This is partly a measurement problem, as only one point out of 1,000 would fall on a vesicle for a rock giving 0.1% vesicles. In practice, counting was continued on the very vesicle-poor rocks until at least one point fell on a vesicle. Because of the log-normal distribution, the logarithmic mean is used as a measure of the vesicularity of a given suite (Table 1). Among lavas collected (and presumably erupted) at about the same depth, the logarithmic mean vesicularity between sample suites varies by an order of magnitude (Figs 1, 2 and Table 1). The extremes in the variation are clearly real and not a result of measurement problems (compare Figs 1 and 2).

The effect of depth of eruption on vesicularity can be partly evaluated by comparing the vesicularity averages with the trend line established independently from samples collected at known depth on the east rift zone of Kilauea volcano and the south rift zone of Mauna Loa volcano, Hawaii. Thirty-five samples were analysed from 16 dredge hauls through a depth range of 490–5,190 m. The least-squares fit of the data on a log plot is shown in Fig. 2. The straight line on the log plot is defined by $\log V = 6.25 - 1.88 \log D$ where V is the volume per cent vesicles and D is the depth in metres. Hence V is very nearly proportional to D^{-2}. Such a relationship is consistent with the increase of V towards shallower depth resulting from both the Boyle's law increase in vesicle size (due to reduction of pressure) and

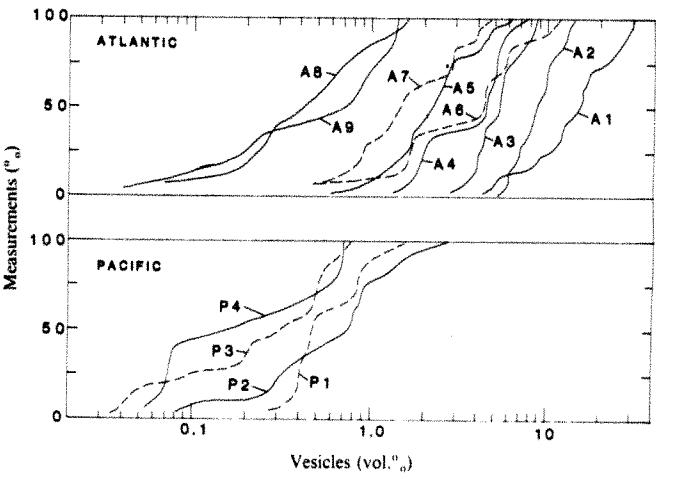


Fig. 1 Cumulative curves of volume per cent vesicles for the outermost 1–1.5 cm of basalt lava flows from 13 ocean ridge segments (see Table 1).

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exsolution of an additional gas phase whose mass is proportional to the reduction in pressure[6]. Therefore, the logarithmic mean vesicularity of sample suites has been projected to a common depth of 3 km parallel to a line with a slope of $V = 1/D^2$ (Table 1, Fig. 2).

The logarithmic mean vesicularity at 3 km ranges from 0.17 to 2.9%, and the Atlantic suites are consistently higher (>0.55%) than those of the Pacific. The adjusted average vesicularity for the Reykjanes Ridge suites (A1, A2, A3) is least reliable because they are shallow and have been extrapolated through the greatest depth range.

The presence of the high-vesicle suites in the Atlantic suggests that they may be favoured by the slower spreading which prevails in the Atlantic. A plot of vesicularity adjusted to 3 km relative to spreading rate (Fig. 3) indicates a rough inverse correlation. Pacific suites range from 0.17 to 0.51% vesicularity and 3 to 8 cm yr^{-1} half spreading rate. Atlantic suites range from 0.56 to 2.9% vesicularity and all spread at about 1 cm yr^{-1}.

Schilling[7] has noted that spreading ridge basalts enriched in the volatile elements Cl, F and Br are also enriched in other incompatible elements, the light rare earth elements (LREE), and show high $^{87}Sr/^{86}Sr$ ratios. The chrondrite normalised ratio La/Sm, which is a measure of LREE enrichment, has been previously determined in rocks from 11 of the 13 segments (see references of Table 1). Samples analysed for rare earth elements are partly the same samples analysed for vesicles, and partly samples which were obtained from localities close to those analysed for vesicles. The LREE enrichment factor (La/Sm)EF shows a moderately good correlation with adjusted vesicularity (Fig. 3). The so-called 'normal ridge basalts' with (La/Sm)EF of about 0.6 show log-mean vesicularities adjusted to 3 km depth <0.7%, and areas enriched in the halogens with higher (La/Sm)EF range in vesicularity up to 2.9% (Fig. 3).

The variation in adjusted vesicularity can be regarded as a measure of the CO_2 content of the rock in excess of saturation as CO_2 is the predominant gas in vesicles. The vesicle CO_2 in the rocks ranges from about 70 p.p.m. for the Galapagos samples (P3) to 1,200 p.p.m. for samples collected from 45° N on the Mid-Atlantic Ridge (A4) assuming a rigid temperature of the basalt glass of 1,000 °C.

The size of the vesicles ranges widely in a single thin section, but vesicles in the matrix glass (excluding glass inclusions in phenocrysts) show a distinct minimum diameter of 3–4 μm. This minimum diameter may be controlled by the surface tension of the melt. The logarithmic median diameter decreases with depth. It is 40–45 μm at 2.5 km depth and 20–25 μm at 5 km depth, but even at this depth, some vesicles attain 100–200 μm in diameter. Hence, a small population of small vesicles must exist in the upper part of magma chambers a few kilometres beneath oceanic spreading ridges.

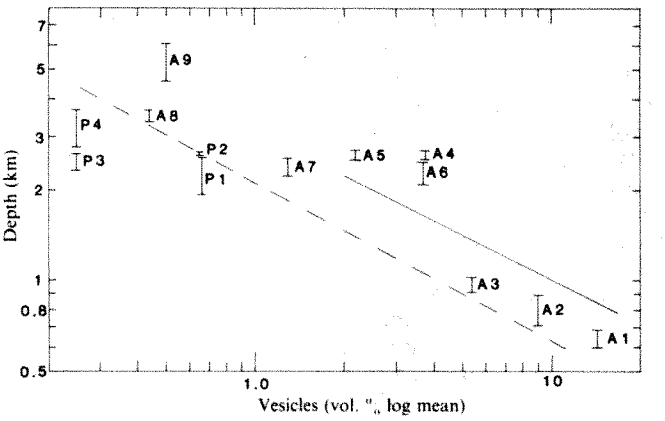


Fig. 2 Logarithmic mean vesicularity relative to depth range for 13 suites of mid-ocean ridge basalt samples. Dashed line is least-squares logarithmic trend line for Hawaiian rift zone samples. All sample suites have been projected parallel to solid line to estimate mean vesicularity at 3 km depth (see Table 1).

Vesicularity controls

The control of the vesicularity of submarine lava is complex because of the transient nature of gas bubbles in melt and the many processes that can operate to enrich, remove, or disperse vesicles. Bubbles tend to rise and concentrate or escape more rapidly than heavy phenocrysts of the same size which would sink because of their greater density contrast with the melt. The processes that control vesicularity may be divided into three general groups depending on their location: (1) during and after eruption on the ocean floor, (2) within magma chambers before

Table 1 Vesicularity of submarine spreading ridge basalts

Symbol	Area	Lat	Long	Depth (m)	No. of measurements	Vesicularity (vol. %) Mean	log median	log mean	log mean/ 3 km	Vesicle CO_2^* (p.p.m.)	Refs
A1	Reykjanes Ridge	61.7–62.8° N	25.2–26.8° W	923–1040	17	16.7	15.0	14.2	0.64	270	2, 8
A2	Reykjanes Ridge	60.0–62.3° N	25.8–29.5° W	710–900	15	9.3	8.5	9.0	0.62	260	2, 8
A3	Reykjanes Ridge	59.9–60.5° N	29.2–29.7° W	600–687	13	5.6	5.4	5.4	0.56	230	2, 8
A4	Mid-Atlantic Ridge	45.2° N	27.9° W	2527–2697	9	4.2	4.5	3.8	2.9	1200	9
A5	Mid-Atlantic Ridge	36.8–36.9° N	33.2–33.3° W	2512–2735	62	2.6	2.4	2.2	1.7	710	4, 10, 11
A6	Mid-Atlantic Ridge	36.7–36.8° N	33.3° W	2100–2500	11	4.9	4.3	3.8	2.3	960	10†
A7	Mid-Atlantic Ridge	36.4–36.5° N	33.6–33.7° W	2230–2560	17	2.0	1.5	1.3	0.80	330	10†
A8	Mid-Atlantic Ridge	23.0–23.2° N	44.9° W	3392–3685	14	0.59	0.41	0.44	0.60	250	12, 13‡
A9	Cayman Trough	18.0–18.7° N	81.7–81.8° W	4610–6142	13	0.70	0·73	0.50	1.6	670	14§
P1	Juan de Fuca Ridge	46.1–46.9° N	129.3–130.1° W	1950–2550	12	0.99	0.77	0.66	0.33	140	15
P2	East Pacific Rise	20.9° N	109.0–109.1° W	2635–2675	7	0.74	0.48	0.65	0.51	210	5
P3	Galapagos Ridge	0.8° N	86.1–86.2° W	2330–2560	19	0.36	0.32	0.25	0.17	70	16‖
P4	East Pacific Rise	27.7–28.1° S	112.9–113.2° W	2800–3713	5	0.37	0.25	0.25	0.28	120	17

* Calculated from log mean vesicularity at 3 km depth assuming all vesicle gas is CO_2 and rigid temperature is 1,000 °C.
Personal communications from: † C. A. Hopson, ‡ F. A. Frey; § J. G. Schilling; ‖ J. B. Corliss.

eruption and within feeding dykes during eruption, and (3) within the source region where magma is generated.

(1) During eruption, the rapid quenching and solidification of pillow basalts on the sea floor is believed to inhibit both the loss of volatiles by bubbles escaping from the lava and the interaction of lava with ocean water. However, recent discoveries on the Galapagos spreading ridge[18] reveal large fluid, non-pillowed lava flows which commonly pond in depressions. Chimneys around the margins of these flows suggest that water trapped beneath was heated and jetted up through the flow, possibly adding to the water content and vesicularity of the flow.

Collapse (or uplift) of the axial zone of a spreading ridge may have occurred after eruption. Such tectonic movements would invalidate the assumption that the depth of collection of submarine lava is the same as the depth of eruption. Collapse of the sea floor from a depth of 2.5 km down to 3.5 km, which is possible on a slow-spreading ridge with a kilometre-deep rift valley, could bring lavas with a vesicularity of 0.9% down to a region where a vesicularity of 0.45% would be reasonably expected.

(2) Since vesicles are ubiquitous in submarine lavas and volatile-rich inclusions are common in phenocrysts, the melts in magma chambers are close to volatile saturation, and some small bubbles are probably present at least in the upper part of shallow magma chambers. If the chamber is non-convecting, such bubbles will tend to rise and collect near the ceiling. If the ceiling is wide and relatively flat, they will be distributed over a wide zone, and most will be incorporated in the solidifying roof rocks marginal to the central region tapped by erupting dykes. If the ceiling is narrow and peaked, then bubbles may accumulate and become enriched in the central apex tapped by dykes.

Other factors might control the distribution of volatiles within the chamber. Processes of liquid diffusion may concentrate volatile components near the cooler ceiling and walls of the chamber or near the ceiling where lower pressure conditions prevail. The content of volatile components in the melt can be enhanced by the digestion and assimilation of hydrothermally altered rocks at the top or upper margins of the chamber and by the crystallisation of early-formed minerals.

The length of feeding dykes, that is, the depth of the magma chamber beneath the ocean floor, will affect the vesicularity of erupted basalt because of the rapid pressure change on eruption. If the volatile content is the same in the upper part of different magma chambers, then lava erupted from a deeper chamber through longer dykes would be more vesicular than that erupted from a shallower chamber, because the greater pressure drop would enhance the growth of existing bubbles and cause exsolution of new bubbles.

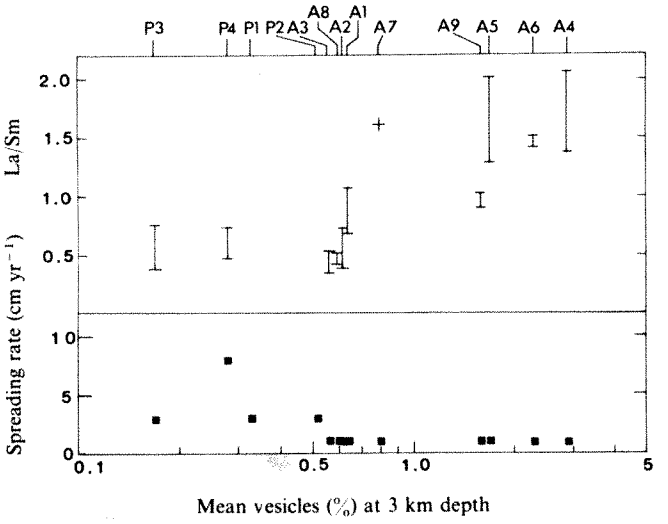


Fig. 3 Logarithmic mean vesicularity adjusted to 3 km depth relative to spreading rate and chrondrite normalised La/Sm ratio for 13 suites of mid-ocean ridge basalt.

(3) The vesicularity of the melt erupted from a magma chamber may be established by differences in the volatile content of magma fed into the bottom of the chamber from the mantle possibly induced by heterogeneities within the mantle, by the mixing of melts from different sources, or by the style of melting.

Discussion

The possibility of lava flows interacting with seawater, thereby increasing vesicularity during eruption and flow on the ocean floor, is believed to be minor because of the general low water content of submarine basalt, which when compared with experimental studies suggests that the melts are undersaturated with water[3]. Moreover, analyses of ridge basalt from four of the regions included in this study (A2, A5, A9, P2) indicate that the predominant vesicle gas is CO_2, not H_2O. Locally, however, water may be added to basalt melt by the chimney activity described by Ballard[18].

The mechanism of collapse of the ocean floor after eruption of the analysed lava cannot be eliminated and indeed tends to increase the vesicularity in the rifted valleys of slow-spreading ridges. However, the anomolously high vesicularity of some of the sample suites cannot be explained by this process unless collapse amounted to 1,400 m (for suites A8 and A4). Furthermore, there is no evidence in the lavas from suite A5 that lavas of different age show a significant difference in vesicularity.

The higher vesicularity of Atlantic spreading ridge basalt suites compared with those from the Pacific (Fig. 3) suggests that some feature of the shallow storage chambers feeding eruptions may control the vesicularity of erupted lava. Pacific spreading ridges generally spread faster (Fig. 3) and consequently the magma chambers are presumably larger and shallower[19,20]. The larger chambers are more likely to contain convecting magma which will inhibit the rise of vesicles and collection near the top. Furthermore, the shallower character of fast-spreading chambers would cause them to be tapped by shorter dykes[20] subjecting the melt to a smaller pressure drop (as compared with the deeper slow-spreading chambers) thus producing less vesicular erupted lava assuming that the tapped melt had the same volatile content. Finally, the narrow and more peaked aspect of slow-spreading chambers may tend to concentrate rising volatiles towards the top centre of the chamber where they will be tapped by dykes and erupted as more vesicular lava on the ocean floor. In the broader flat-topped chambers of fast-spreading ridges, volatiles will be concentrated over a broader zone of the ceiling, where a higher proportion will be incorporated in the solidifying roof rocks marginal to the fluid central zone tapped by erupting dykes.

Such an explanation cannot account for differences in the vesicularity of Atlantic suites, all of which occur where spreading rates are low and close to a half rate of 1 cm yr^{-1}. However, Cann[20] has suggested that because spreading rates of much less than 1 cm yr^{-1} have not been found, this may mark a lower limit of spreading, where the magma chamber is very narrow[19] and perhaps discontinuous along strike and through time. In such chambers, volatile concentrations of erupted lava may be especially enriched in favourable places by the processes discussed.

The vesicularity contrasts may also be caused by differences in the composition of magma fed into magma chambers from the mantle. Schilling and coworkers[7,8] have amassed data showing that significant concentrations of trace elements occur in ocean ridge basalts from certain areas where magmas are apparently derived from deep mantle plumes. Such plume-derived lavas are enriched in the halogens, LREE, $^{87}Sr/^{86}Sr$, and other incompatible elements of varying volatility, including K, Ba, Rb and Cs. In the Atlantic, such plume areas include Iceland (A1) and the Azores region (A4, A5, A6, A7), which this study demonstrates are also enriched in vesicles and CO_2. Hence the plume areas may represent sites of upwelling of partial melt from a deep mantle source relatively undepleted in volatiles, and other incompatible, large ion elements. In contrast, the basalts of

so-called normal ridge segments such as A8 and the Pacific suites that were sampled are derived from a shallower mantle zone previously depleted in these constituents.

CO_2 is the primary discrete volatile phase in melts erupted on the ocean floor and no doubt exists also as a discrete phase as bubbles in subjacent magma chambers, where it is commonly entrapped in phenocryst minerals. Hence spreading ridge magma chambers which may exist for millions of years will be subjected to a constant flux of upward-streaming CO_2. The extent to which this CO_2 is concentrated in favourable places and is able to concentrate other trace elements and affect isotopic ratios is poorly known. Much of the evidence suggests that the unique chemistry of plume-type basalt is derived from a different, deeper mantle source. But the high CO_2 content of these basalts indicates that their composition may have been substantially altered by the CO_2 flux.

It is clear from Fig. 2 that high-vesicle lavas have a much better potential for building islands than do low-vesicle lavas. If we contrast extreme areas with an adjusted average vesicularity at 3 km of 2% with one of 0.30%, then projecting parallel to the Kilauea curve, these values increase at 2 km depth to 4.4 and 0.65%, and at 1 km depth to 17 and 2.5%. If the same amount of melt was erupted from each centre, the high-vesicle lava would build up more rapidly and its rate of buildup would accelerate more rapidly. Hence the presence of islands such as the Azores and Iceland near regions of high-volatile lavas may partly result from this process.

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Biomass burning as a source of atmospheric gases CO, H_2, N_2O, NO, CH_3Cl and COS

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Biomass burning can contribute extensively to the budgets of several gases which are important in atmospheric chemistry. In several cases the emission is comparable to the technological source. Most burning takes place in the tropics in the dry season and is caused by man's activities.

THE potential importance of deforestation and biomass burning for the atmospheric CO_2 cycle has received much attention and caused some controversy[1–3]. In this article we will show the probable importance of biomass burning as a trace gas source, which is caused by man's activities in the tropics. We used the results of our global biomass burning analysis[4] to derive some rough estimates of the sources of the important atmospheric trace gases CO, H_2, CH_4, N_2O, NO_x (NO and NO_2), COS and CH_3Cl from the worldwide burning of biomass. Table 1 shows the results of our study for different activities and ecosystems.

Our approach has been to relate the emission quantities of these gases to those of CO_2 in fire plumes. We have determined such emission ratios during two major forest fires. The first was from a forest fire ~12.5 km south-west of Meeker, Colorado. The crown fire was fuelled by pinon and juniper timber, but most of the material burned consisted of annual and perennial grasses, shrub juniper, and sagebrush in the undergrowth. The dense plume rose to a height of ~4 km before flattening and moving horizontally. Samples were collected in stainless steel containers during flights through the smoke plume at different altitudes. Background samples were also collected, at plume altitude, 6 km before reaching the sampling area and again when leaving the fire area.

The second fire occurred in the Wild Basin area of the Rocky Mountain National Forest, not far from the Meeker site, in a mature spruce and fir forest in which there was much dead woody material on the ground and much less green vegetation than in the Meeker fire. In this case samples were taken on ground level. CO_2, CO, CH_4, H_2, COS and CH_3Cl were analysed by gas chromatography; N_2O was determined by mass spectrometry. To check for nonlinear gas-chromatographic responses at high concentrations in the samples, a volumetric measurement of CO_2 was also performed.

Some information on emissions from wood burning can also be derived from earlier field and laboratory experiments[5–9]. the emission ratios are shown in Table 2 which includes only collections with CO_2 concentrations at least 10% above background. At this stage we have not been able to measure gaseous emissions in tropical ecosystems. This is clearly a disadvantage, but it should not invalidate our main conclusions. Biomass burning has previously been considered unimportant as a global source of atmospheric trace gases[10]— our analysis shows that this is not the case.

Importance of trace gases in the atmosphere

Some of the trace gases such as CO_2, N_2O and CH_4 considered in this study contribute to the atmospheric greenhouse effect by their absorption of terrestrial thermal radiation[11].

Other trace gases such as carbon monoxide (CO) strongly affect the tropospheric concentrations of the highly reactive

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hydroxyl (OH) radical through the reaction

$$CO + OH \rightarrow CO_2 + H \quad (1)$$

It has, therefore, been proposed that a growth in tropospheric CO from industrial activities would lead to a decrease in tropospheric OH concentrations[12]. As the only significant tropospheric sink for many gases (especially hydrocarbons and chlorinated hydrocarbons) is reaction with OH, a rise in the tropospheric CO content would cause enhanced concentrations of these gases in the troposphere and a greater transfer to the stratosphere, with possible effects on stratospheric ozone. Other gases which affect the concentration of OH in the stratosphere include methane (CH_4) and molecular hydrogen (H_2).

Although the oxidation of methane (CH_4) is important, molecular hydrogen (H_2) can also add to the reservoir of stratospheric water vapour. This will affect the formation of stratospheric OH, with further consequences on the chemical and thermal balance of the stratosphere[13].

Little of the nitric oxide (NO) emitted at ground level can reach the stratosphere because of the efficient precipitative scavenging of its oxidation products NO_2 and HNO_3. The presence of NO in the troposphere affects the formation of ozone, through the oxidation of carbon monoxide, methane and other hydrocarbons[14]. It is therefore conceivable that increasing industrial inputs of CO and NO in the atmosphere will lead to increasing levels of tropospheric ozone[14].

As nitrous oxide (N_2O) does not react in the troposphere, it will have a long tropospheric residence time and reach the stratosphere where its oxidation leads to the formation of NO and NO_2. These gases appear in catalytic cycles of reactions leading to less ozone above 25 km and more ozone below this altitude[15].

Carbonyl sulphide (COS) and methyl chloride (CH_3Cl) have similar importance in stratospheric chemistry. During periods of little volcanic activity it is possible that the photodissociation of COS is the main source of sulphur in the stratospheric sulphate layer. Although little is known about the sources of COS there may be a growing anthropogenic input of it[16]. The decay of CH_3Cl in the stratosphere releases Cl and ClO, which are extremely efficient in limiting stratospheric ozone by catalytic reactions. An important source of methyl chloride is its emanation from the oceans, but its production by biomass burning has also been proposed[17].

Global source estimates

The data collected in Table 2 show an encouraging uniformity. Individual average emission ratios for several gases do not deviate more than a factor of 6 from each other. The largest variations in emission ratios are found for N_2O, COS and CH_3Cl. This is not surprising as one may expect these emissions to be particularly dependent on the type of material being burned. For example, the relative nutrient content in the green parts of the biomass is much larger than in stemwood[18]. We are not aware of any published emission data for the gases N_2O, H_2, CH_3Cl and COS. With the exception of the lower values obtained by Darley *et al.*[5] and Boubel *et al.*[7], the CO/CO_2 emission ratios have all been determined to be in the range of 12–20%.

Because of the uniform CO/CO_2 ratios reported, we can give a reasonable indication of the magnitude of the CO emissions. The same may apply to CH_4 and H_2. It is clear, however, that any estimations are much less reliable for NO_x, N_2O, COS and CH_3Cl. However, we can still indicate the potential importance of biomass burning as a source of these gases to the atmosphere.

If the data on total worldwide CO_2 emissions from wood burning of $2–4 \times 10^{15}$ g C yr^{-1} from Table 1 are accepted, we can discuss its possible implications for the budgets of CO, H_2, CH_4, NO_x, N_2O, CH_3Cl and COS. The wood burning contribution can then be compared with previously derived estimates of the sources of these gases from other anthropogenic or natural processes. Note that our estimates on the global extent of biomass burning are substantially smaller than those of other workers such as Wong[2] and Woodwell *et al.*[3]. We cannot defend the data compiled in Table 1 here due to lack of space, however, acceptance of higher biomass burning rates will clearly lead to larger estimates of trace gas emission rates.

Adopting an average relative CO/CO_2 volume emission factor of 14% from the data collected in Table 2, the average estimated input of CO would be 8.4×10^{14} g CO yr^{-1}, with a range of about $2.4–16.6 \times 10^{14}$ g CO yr^{-1}. Seiler[19] has estimated that the technological input of 6.4×10^{14} g CO yr^{-1} is the dominant source of atmospheric CO, and a global CO emission of 2.9×10^{14} g yr^{-1} from burning has been estimated by Bach[20]. Clearly, the CO input form fires is probably also important for the global CO budget and for the interpretation of the global CO distribution.

With a relative H_2/CO_2 emission ratio of 3.3%, the production of H_2 would amount to 15×10^{12} g H_2 yr^{-1}, with an estimated range of $9–21 \times 10^{12}$ g H_2 yr^{-1}. Schmidt[21] estimated the total worldwide source of H_2 to be 36.5×10^{12} g yr^{-1}, including an anthropogenic contribution of 25.5×10^{12} g yr^{-1}. Although our analysis shows that fires are probably important no such contribution was considered in his budget.

Assuming an average CH_4/CO_2 emission ratio factor of 1.6%, the global source of CH_4 can be calculated as

Table 1 Summary of data for the annually burned area and biomass

Activity	Burned and/or cleared area	Total biomass cleared	Biomass exposed to fire	Annually burned biomass	Dead below-ground biomass	Dead unburned above-ground biomass
Burning due to shifting agriculture	21–62 (41)	31–92 (62)	24–72 (48)	9–25 (17)	7–20 (14)	16–72 (44)
Deforestation due to population increase and colonisation	8.8–15.1 (12.0)	20–33 (26.5)	16–25 (20.5)	5.5–8.8 (7.2)	4.0–8.0 (6.0)	10.5–16.0 (13.3)
Burning of savanna and bushland	(600)		12.2–23.8 (18)	4.8–19 (11.9)	8–16 (12)	2.4–4.8 (3.6)
Wildfires in temperate forests	3.0–5.0 (4.1)	10.5–17.5 (14.0)	7.7–12.8 (10.3)	1.5–2.6 (2.1)	2.8–4.7 (3.8)	6.2–10.2 (8.2)
Prescribed fires in temperate forests	2.0–3.0 (2.5)	1.2–1.8 (1.5)	0.3–0.5 (0.4)	0.1–0.2 (0.2)	0.6–0.9 (0.8)	0.2–0.3 (0.3)
Wild fires in boreal forests	1.0–1.5 (1.3)	2.5–3.8 (3.2)	1.8–2.7 (2.3)	0.4–0.6 (0.5)	0.7–1.1 (0.9)	1.4–2.1 (1.8)
Burning of industrial wood and fuel wood		31–32 (31.5)	11–12 (11.5)	10–11 (10.5)	5.5	1*
Burning of agricultural wastes			19–23 (21)	17–21 (19)	27–31 (29)	1.9–2.3 (2.1)
Total	630–690 (660)	130–250 (180)	92–172 (132)	48–88 (68)	56–87 (72)	40–109 (74)

Units 100 Tg dry matter and 10^6 hectares; to convert dry matter to carbon multiply by 0.45. Data in parentheses represent average values.
* Excluding wood used in long lasting structures.

Table 2 Compilation of product yields by volume relative to that of CO_2 from different studies

Excess of trace gas concentrations compared to excess CO_2 over background	H_2 (%)	CH_4 (%)	CO (%)	N_2O (%)	NO_x (%)	COS ($\times10^{-6}$)	CH_3Cl ($\times10^{-6}$)
Meeker forest fire (12 July 1977)							
Average (four observations)	3.3	2.1	12.4	0.38			
Range	2.9–3.5	1.6–2.5	11.2–13.5	0.23–0.50			
Wild Basin fire (20 September 1978)							
Average (seven observations)		2.2	19.9	0.06		15.8	23.4
Range		1.0–3.4	15.8–25.1	0.02–0.08		5.4–28.6	4.4–57.2
Agricultural wastes[5]		1.2	6.2				
		0.15–4.6	3.3–15.8				
Landscape refuse[6]			14.6		0.28		
Grass, stubble and straw[7]							
Average			6.3				
Range			2.9–14.9				
Eight pine slash fires[8] (average)			19.7		$0.65(NO+NO_2)$		
Wood burning in fire places[9] (average)			18.0				
Average ratios used for source estimates	3.3	1.6	14	0.22	0.47	15.8	23.4
Range	2.9–3.5	1.0–2.2	5.5–21.6	0.02–0.50	0.28–0.65	5.4–28.6	4.4–57.2

The forest fire plume data were obtained from wild fire observations. The remaining data were obtained from controlled fires or laboratory measurements.

6×10^{13} g yr^{-1} with a range of $2.5–11\times10^{13}$ g CH_4 yr^{-1}. The combustion vegetation could contribute to the CH_4 budget of the atmosphere, which has an estimated input of $40–83\times10^{13}$ g CH_4 yr^{-1} (ref. 22). However, methane is not the only hydrocarbon gas emitted from the burning of vegetation: emissions of many reactive hydrocarbons, including acetylene (C_2H_2) have also been detected[5] and for some of these gases wood burning may be an important source.

As explained above, the derivation of global source estimates for N_2O, NO_x, COS and CH_3Cl is not trivial. The resulting fluxes which we will derive for these gases are, therefore, less reliable than those derived for CO, H_2 and CH_4. The simplest way of estimating the flux would be to apply the same procedure as for CO, H_2 and CH_4. With an approximate N_2O/CO_2 emission factor of 0.22%, the estimated nitrous oxide source would be 20×10^{12} g N_2O yr^{-1}. Similar calculations would yield a source of 14×10^{12} g N yr^{-1} for NO_x, 1.1×10^{11} g S yr^{-1} for COS, and 1.9×10^{11} g Cl yr^{-1} for CH_3Cl.

Although this approach indicates the potential importance of emission strengths, it is a dubious estimation. An upper limit to the NO_x emissions can be obtained by estimating the mass of bound nitrogen in the burned plant material and by assuming that almost all bound nitrogen is emitted as NO_x—an assumption consistent with other experimental studies[8,23,24]. The average of eight experimental slash fires gave relative volume emission ratios NO_x/CO_2 of 4% for duff and 2.1% for needles. These ratios are close to the values that can be expected if essentially all bound nitrogen in the plant material were volatilised as NO_x. No NO_x emissions were detected during the combustion of cellulose materials, presumably because burning temperatures were too low to allow appreciable NO_x production from atmospheric N_2 and O_2. The relative volume yield NO_x/CO_2 from an entire fire (in which about 55% of all available fuel was consumed) was estimated to be 0.65%[8]. In living plants the nutrients N and S are mainly concentrated in those parts which are most easily burned, such as leaves, small twigs and bark. Before burning, however, much plant tissue is dry and dead, and has actually lost nutrients. Using the information in our Table 1 and Tables 53 and 54 in the compilation by Rodin and Bazilevich[18], the average maximum global N/C ratio in the burned biomass can be roughly estimated to be between 1.5 and 2.5%. Given this information we derive a maximum global average NO_x emission of 50×10^{12} g N yr^{-1} from biomass burning, with a range of $20–100\times10^{12}$ g N yr^{-1} if most bound nitrogen is indeed emitted as NO_x. The mean estimate is about equal to that mentioned previously by Delwiche and Likens[25].

The assumption that all fixed nitrogen is released as NO_x is far from established. A study by Clements and McMahon[26] confirms that most of the NO_x released during burning arises from fuel nitrogen. They conclude that environmentally significant amounts of NO_x are formed by the burning of forest fuels. However, this study also indicated that the NO_x yield is not 100% and that it actually increases with the nitrogen content of the fuel: a rough average of the NO_x yield observed in their studies is about 30%. From a mass-balance perspective it is therefore conceivable that other trace gases besides NO_x are released by biomass burning. These could include N_2O, NH_3 and HCN. NH_3 is an important gas involved in the atmospheric nitrogen cycle. HCN has not so far been observed in the atmosphere, but it could be rather stable as it is not removed photochemically in the troposphere.

If we assume that our maximum observed N_2O/CO_2 emission ratio of ~0.4% is produced from the burning of the nutrient-rich portions of the vegetation (such as the needles and leaves, which have an average N/C composition ratio of 4–6%), we may guess that the N_2O volume emissions could be ~8% of those of NO_x. Consequently we may estimate an average global N_2O source of $\sim13\times10^{12}$ g N_2O yr^{-1}. Finally, comparing the relative emission ratios of COS and CH_3Cl to those of N_2O obtained from the Wild Basin fire in Colorado, the global emission rate of COS can be roughly extrapolated to be 2.4×10^{11} g S yr^{-1} (3×10^7 molecules cm^{-2} s^{-1} at the Earth's surface) and that of CH_3Cl to be 4.2×10^{11} g Cl yr^{-1} ($\approx4\times10^7$ molecules cm^{-2} s^{-1}). Judging from these estimates, biomass burning could produce much more COS than is destroyed in the stratosphere[16]. This may indicate the presence of another sink for COS in the troposphere or at the Earth's surface. On the other hand, the production of CH_3Cl from biomass burning seems to be an order of magnitude lower than its destruction in the troposphere by reaction with OH[27]. Therefore, from this limited analysis it seems that the oceanic source of CH_3Cl should be more important[17].

The indicated emissions of NO_x and N_2O both seem very important from a global perspective. A NO_x source strength of several times 10^{13} g N yr^{-1} is comparable to or larger than the other two major sources of NO_x (industrial processes[28] and lightning[29]). The production of NO_x by the burning of vegetation during the dry season has been clearly demonstrated by Lewis and Weibezahn[30], who measured an increase in rainfall acidity by 10^4 from the beginning to the end of the dry season in the Aragua Valley in Venezuela. According to these authors the release of NO_x by seasonal vegetation burning and its conversion to HNO_3 were responsible for this phenomenon. The S/N ratios in plant material are too low for H_2SO_4 to be nearly as important as HNO_3 in the acidification of the precipitation. The observations by Lewis and Weibezahn[30] supported our method of extrapolation from mid-latitude to tropical conditions.

The indicated production rates of N_2O by burning are comparable to the removal rate in the stratosphere, which is still

the only well-documented sink for nitrous oxide. The large oceanic source of N_2O once derived by Hahn[31] is now strongly disputed and more recent observations point to a much smaller source[32,33]. It seems, therefore, that vegetation burning as a source of N_2O could be of a similar substantial importance.

Note that in our source calculations for the atmospheric trace gases, we did not consider the potential release from the heating of the topsoil organic matter or from the $40–80 \times 10^{14}$ g C of matter which is exposed to fire but left behind as dead, unburned above-ground biomass (see Table 1). The topsoil organic matter is especially rich in nutrients and may make imporant contributions to the cycling of atmospheric trace gases and nutrient elements. The latter has already been pointed out by Lewis[34].

The analysis of emissions from the burning of vegetation has also revealed substantial yields of aldehydes, hydrogen peroxide (G. Kok, personal communication), and reactive and oxygenated hydrocarbons[7,35,36]. Because of the simultaneous production of NO, there may be a significant production of ozone in the fire-affected air. Such production of ozone has been observed[37].

Despite the limited amount of observations, we must conclude that biomass burning, especially in the tropics where the rates of biomass production and biomass burning are unparalleled, has the potential to contribute in an important way to the global budgets of several major atmospheric trace gases. We also need to consider that tropical emissions occur in a photochemically very active and dynamically important region, in which substantial transfer of tropospheric air to the stratosphere takes place.

Observations of biomass burning in the tropics are needed which will enable us to assess far more reliably the role of biomass burning in biogeochemical cycles and in atmospheric chemistry. This research is especially important in view of the increasing use of forest resources in the developing countries.

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A modified RNA polymerase transcribes a cloned gene under sporulation control in *Bacillus subtilis*

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A modified form of RNA polymerase from Bacillus subtilis *selectively transcribes a cloned gene under early sporulation control. This RNA polymerase lacks σ factor but contains a newly identified subunit of molecular weight 37,000, termed P^{37}. P^{37} could be a regulatory protein that controls, at least in part, an early stage of spore development.*

THE formation of dormant endospores in *Bacillus subtilis* is a simple developmental process that proceeds through a series of well defined morphological stages (for reviews see refs 1, 2). During the earliest developmental stages, the sporulating cell partitions itself by invagination of the plasma membrane into a forespore compartment and a mother cell or sporangium. Then, after engulfment of the forespore by the mother cell membrane, the developing spore acquires a cortex and a tough protein coat. Finally, a mature, dormant spore is liberated by lysis of the mother cell. Mutations in a variety of sporulation or *spo* genes arrest this process at different stages of spore development[1].

What mechanisms govern the expression of sporulation genes during this developmental sequence? To help answer this question, this laboratory previously cloned a *B. subtilis* gene whose transcription is activated at an early stage of spore development[3]. This gene, which maps near the origin of replication of the *B. subtilis* chromosome (between *spo0J* and *spo*IIE20, ref. 4, and W.G.H., C. Banner, J. F. Ollington, R.L., and A. L. Sonenshein, unpublished results) encodes an RNA of about 400 bases and referred to as the '0.4-kilobase gene'. This gene is known to be under sporulation control because its transcription is severely restricted in mutants blocked at the earliest stage of development (*spo0A*, *spo0B*, *spo0E*, *spo0F* and *spo0H* mutants; ref. 3 and unpublished results). Here we report the isolation from early sporulating cells of a form of *B. subtilis* RNA polymerase that transcribes this developmentally regulated gene *in vitro*.

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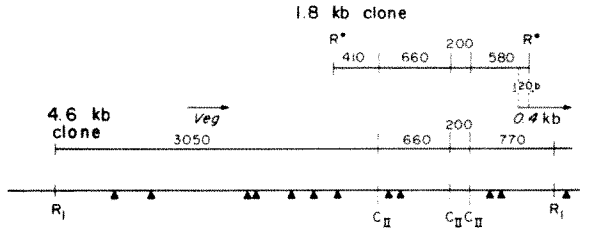


Fig. 1 Endonuclease restriction map of cloned *B. subtilis* DNA in the vicinity of the 0.4-kilobase gene. The lower line is a map of the cleavage sites for the restriction enzymes *Hpa*II (▲), *Eco*RI (as indicated) and *Hin*cII (as indicated) and is a composite of the maps of several overlapping cloned DNAs. The middle line is a map of one cloned DNA of 4.6 kilobases that terminates at an *Eco*RI site within the 0.4-kilobase gene, and the top line is a map of a cloned 1.8-kilobase DNA that terminates at an *Eco*RI* cleavage site within the 0.4-kilobase gene. The two heavy arrows show the location and direction of transcription of the 0.4-kilobase gene and the vegetative (*veg*) gene. The numbers on the middle and top lines show the size in base pairs of fragments generated by *Hin*cII cleavage of the 4.6- and 1.8-kilobase DNAs (see Figs 2, 4). The dashed lines show the position of the 120 base run-off RNA (see Fig. 4). Documentation for the information in this figure will be presented elsewhere (J. F. Ollington, W.G.H. and R.L., manuscript in preparation).

Detection of a specific transcribing activity

Figure 1 presents an endonuclease restriction map of cloned *B. subtilis* DNA in the vicinity of the 0.4-kilobase gene. The 0.4-kilobase gene, as depicted, is transcribed from left to right with its startpoint for transcription located about 300 base pairs from an internal *Eco*RI cleavage site (J. F. Ollington, W.G.H. and R.L., in preparation). At 2.7 kilobases to the left of the 0.4-kilobase gene, there is a sequence that is transcribed during growth and sporulation and referred to as the 'vegetative' gene.

We devised a specific hybridisation assay to search for an activity that would transcribe the 0.4-kilobase gene. A hybrid plasmid containing a 4.6-kilobase insert defined by the two *Eco*RI sites in the map was cleaved with the restriction enzymes *Hin*cII and *Eco*RI. This generated fragments of 3,050 and 2,200 base pairs that contained the vegetative gene and a 770-base pair fragment containing the promoter-proximal portion of the 0.4-kilobase gene as well as several other fragments of *B. subtilis* and vector DNA. (The 2,200-base pair DNA is a secondary cleavage product of the 3,050-base pair fragment that is generated from an *Eco*RI* site near the left end of the 4.6-kilobase DNA; see Fig. 2 legend). These fragments were separated electrophoretically and transferred to nitrocellulose by the method of Southern[5]. Radioactive RNA from cells pulse-labelled early in sporulation hybridised to fragments containing the vegetative gene and the 0.4-kilobase gene (ref. 3 and J. F. Ollington, W.G.H. and R.L., in preparation). In contrast, radioactive RNA from several different stage 0-blocked mutants annealed only to 3,050- and 2,200-base pair fragments containing the vegetative gene.

To test for transcription of the 0.4-kilobase gene *in vitro*, we hybridised radioactive RNA copied from hybrid plasmid DNA to the electrophoretically separated fragments. The experiment of Fig. 2 shows that RNA polymerase holoenzyme (lane 1) and core RNA polymerase (containing a trace of σ polypeptide) purified from vegetative cells (lane 2) selectively transcribed the vegetative gene region of the hybrid plasmid although neither form of polymerase copied RNA from the 770-base pair region that contained the 0.4-kilobase gene. Thus, purified vegetative RNA polymerase failed to recognise the sporulation-controlled sequence.

To search for a form of RNA polymerase that would copy the 0.4-kilobase gene, we examined extracts of cells collected early (T_1) in sporulation. We identified such an activity in enzyme partially purified by phase partitioning, gel filtration and phosphocellulose chromatography. Lane 3 of Fig. 2 shows that partially purified sporulation enzyme actively copied RNA from the 770-base pair region as well as from the vegetative gene-containing fragments. Some transcription of two plasmid vector fragments (2,400 and 2,210 base pairs) could also be detected. Transcription of hybrid plasmid DNA was not random, as we could detect no hybridisation to the 660- and 200-base pair fragments preceding the 0.4-kilobase gene or to a vector fragment of 950 base pairs.

As a further test of recognition of the 0.4-kilobase gene, we examined the strand selectivity of transcription from the 770-base pair region. DNA strands of the 770-base pair fragment were separated by gel electrophoresis of denatured DNA and then transferred by the Southern[5] procedure to nitrocellulose. Hybridisation of radioactive 0.4-kilobase RNA synthesised *in vivo* identified the slow-migrating DNA as the DNA coding strand (Fig. 3, lane 3). (Other work showing that the 5'-end of the slow-migrating strand is at the *Eco*RI end of the 770-base pair fragment established the direction of transcription as shown in Fig. 1). Figure 3 (lane 2) shows that RNA synthesised *in vitro* from hybrid plasmid DNA annealed selectively to the slow-migrating strand. Combining this result with the result of Fig. 2, we conclude that the RNA polymerase activity from early sporulating cells initiated transcription within the 770-base pair region and copied RNA asymmetrically from the coding strand of the 0.4-kilobase gene.

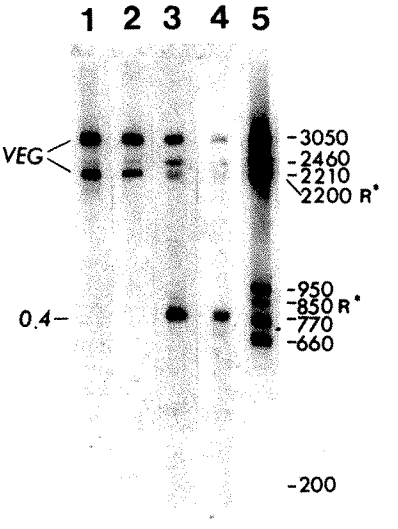


Fig. 2 Southern hybridisations to electrophoretically separated restriction fragments of hybrid plasmid DNA. A pMB9 hybrid plasmid containing the 4.6-kilobase insert of *B. subtilis* DNA[3] described in Fig. 1 was cleaved with the restriction enzymes *Eco*RI and *Hin*cII, generating plasmid vector fragments of 2,460 2,210 and 950 base pairs and *B. subtilis* DNA fragments of 3,050, 770, 660 and 200 base pairs. (In addition, *Eco*RI cleavage generated small amounts of two fragments of 2,200 and 850 base pairs that arise by secondary cleavage of the *B. subtilis* 3,050-base pair fragment at an *Eco*RI* site located 850 base pairs from the left end of the 4.6-kilobase DNA. *Eco*RI* is an altered form of *Eco*RI of reduced recognition specificity[11].) The fragments were separated electrophoretically and transferred to nitrocellulose by ascending chromatography as described by Southern[5]. The nitrocellulose strips (0.5 μg DNA per strip) were then incubated in hybridisation conditions (5 × SSC, 50% formamide; 18 h at 37 °C) with radioactive RNA that had been copied *in vitro* from hybrid plasmid DNA. RNA was synthesised *in vitro* by the following protocol: 0.1 units [a unit incorporates 1 nmol of AMP into RNA with poly(dA-T) as template] of RNA polymerase holoenzyme[12] (lane 1), core RNA polymerase[12] (lane 2), phosphocellulose-purified sporulation polymerase (fraction 20 of Fig. 5; lane 3) or DNA-cellulose-purified sporulation polymerase (fraction 36 of Fig. 6, lane 4) were separately allowed to pre-bind for 10 min at 37 °C with 2.5 μg of pMB9–4.6-kilobase hybrid plasmid DNA (representing an enzyme to template ratio of about 0.5–1.0) in 50-μl reaction mixtures[6] lacking ribonucleotides but containing 20% glycerol. Next, ribonucleotides (0.15mM CTP, GTP and ATP and 0.4 μm (5 μCi) [α^{32}P] UTP) were added. After 4 min incubation, unlabelled UTP (0.15 mM) was added. The reactions were stopped after 2 min and RNA was isolated for hybridisation as described elsewhere[6]. After the hybridisation reactions were completed, the Southern strips were washed, treated with RNase and exposed to X-ray film (Kodak SB-5) to visualise hybrids. Lane 5 shows the position of plasmid vector and *B. subtilis* DNA fragments as visualised by annealing a nitrocellulose strip (that had been treated by the Denhardt[13] procedure) with hybrid plasmid DNA that had been radioactively labelled *in vitro* by the 'nick-translation' activity[14] of DNA polymerase I.

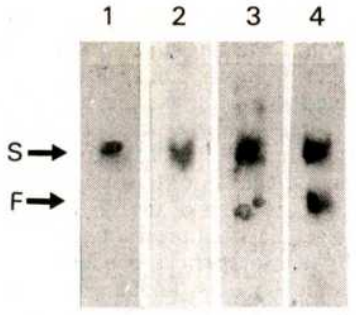


Fig. 3 DNA strand selectivity of transcription. The 770-base pair fragment (containing the 0.4-kilobase gene) was isolated by electrophoresis of pMB9–4.6-kilobase hybrid plasmid DNA that had been cleaved with *Hin*cII and *Eco*RI (see Fig. 1). The strands of the 770-base pair fragment were then separated by electrophoresis of denatured DNA through a 5% polyacrylamide gel as described by Hayward[15]. The separated DNA strands (0.05 μg per strip) were then transferred by the Southern[5] procedure to nitrocellulose. Nitrocellulose strips containing separated DNA strands were then incubated in hybridisation conditions (see Fig. 1 legend) with: lane 1, 120-base run-off RNA from the experiment of Fig. 4; lane 2, RNA transcribed *in vitro* from pMB9–4.6-kilobase hybrid plasmid DNA by phosphocellulose-purified sporulation polymerase from the experiment of Fig. 2; lane 3, *in vivo* synthesised 0.4-kilobase RNA isolated from ^{32}P-labelled sporulating cells as described previously[3]. After hybridisation, the strips were washed, treated with RNase and exposed to X-ray film to visualise hybrids. Lane 4 shows the position of the slow-migrating (S) and fast-migrating (F) strands as visualised by annealing a nitrocellulose strip with radioactively labelled hybrid plasmid DNA (see Fig. 2 legend).

Initiation of transcription at a unique site

Does sporulation enzyme initiate transcription at a unique site within the 770-base pair region? To answer this question we used a cloned subfragment of 1.8 kilobases that contained the promoter-proximal region of the 0.4-kilobase gene and entirely lacked the vegetative gene. The right-hand end of the 1.8-kilobase fragment is an *Eco*RI* cleavage site that falls within the 0.4-kilobase gene at 100–150 base pairs from the startpoint of transcription. We therefore reasoned that, if polymerase was initiating at a unique site near the promoter for the 0.4-kilobase gene, the RNA polymerase from sporulating cells with 1.8-kilobase DNA as template should generate a discrete-sized 'run-off' RNA of 100–150 bases.

This expectation was confirmed by the finding, shown in Fig. 4, that sporulation enzyme generated an RNA of 120 bases from hybrid plasmid DNA that had been cleaved with restriction enzyme to free the 1.8-kilobase insert from the vector. The size of this transcript was apparently dependent on run-off termination at an end of the cut DNA template, as the 120-base RNA was not generated during transcription of uncut plasmid template (Fig. 4). To prove that the 120-base RNA was copied from the 0.4-kilobase region of the hybrid plasmid, *in vitro* synthesised transcript was eluted from a slice of polyacrylamide gel containing the RNA band and from surrounding slices and annealed to electrophoretically separated fragments of the hybrid plasmid. Figure 4*b* shows that hybridisation to the 580-base pair (Fig. 1) fragment containing the 0.4-kilobase gene promoter region was only observed for the slice that contained the 120-base RNA. Finally, hybridisation to separated DNA strands (Fig. 3, lane 1) showed that the 120-base RNA was complementary to the DNA coding strand for the 0.4-kilobase gene. Thus, we conclude that sporulation enzyme initiated transcription at a unique site close to or identical with the promoter for the 0.4-kilobase gene.

Isolation of a modified RNA polymerase

Using cloned DNAs as probes for specific RNA synthesis, we extensively purified a modified form of RNA polymerase that transcribes the 0.4-kilobase gene. Figure 5*a* shows the polypeptide profile of proteins eluted from phosphocellulose with a linear salt gradient. As judged by hybridisation of *in vitro* synthesised RNA to the 770-base pair fragment (Fig. 5*b*) and by the production of the 120-base RNA (Fig. 5*c*), transcription of the 0.4-kilobase gene was preferentially associated with RNA polymerase eluting just past the centre of the gradient at 0.43 M KCl.

Although RNA polymerase was impure at this stage, the subunits of core RNA polymerase ($\beta'\beta\alpha_2$) were readily visualised in fractions across the gradient. In addition, those fractions that transcribed the 0.4-kilobase gene seemed to contain preferentially polypeptides of molecular weight (MW) 37,000 (P^{37}) and 36,000 (P^{36}).

Fractions 20–22 (containing RNA polymerase that preferentially transcribed the 0.4-kilobase gene) were pooled and subjected to DNA-cellulose chromatography. Gradient elution resolved two forms of RNA polymerase (Fig. 6*a*). In addition to the subunits of core RNA polymerase, proteins eluting at lower ionic strength (0.30 M KCl) included the P^{36} polypeptide and a

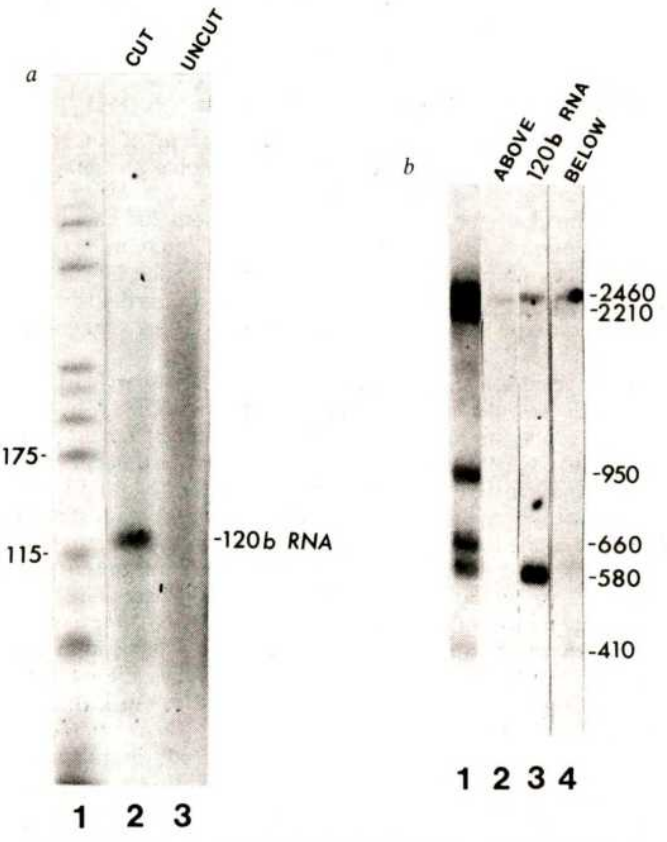


Fig. 4 Synthesis of a 120-base run-off RNA. *a*, RNA was copied *in vitro* using 0.2 units of phosphocellulose-purified sporulation polymerase (fraction 20 of Fig. 5) from a pMB9 hybrid plasmid containing the 1.8-kilobase *Eco*RI* fragment described in Fig. 1 legend. The conditions of RNA synthesis were as described in Fig. 2 legend except that heparin (30 μg) was added 30 s after the addition of ribonucleotides to block re-initiation of transcription. The template (2.5 μg) for *in vitro* transcription was either uncut hybrid plasmid DNA (lane 2) or hybrid plasmid DNA that had been cleaved with *Eco*RI to separate the 1.8-kilobase insert from vector DNA (lane 3). (Even though the 1.8-kilobase DNA was defined by two *Eco*RI* restriction sites, when cloned into the *Eco*RI site of pMB9, the resulting *Eco*RI–*Eco*RI* hybrid site was readily cleaved in *Eco*RI conditions.) *In vitro* synthesised RNA was then subjected to electrophoresis through a 5% polyacrylamide gel containing 7 M urea. As MW standards, lane 1 displays denatured *Hae*III fragments of ^{32}P-labelled pMB9 DNA. The numbers on the left of *a* give the size in bases of two fragments. After electrophoresis, the polyacrylamide gel was exposed to X-ray film (Kodak SB-5) to visualise *in vitro* synthesised RNA and the radioactive standards. *b*, After visualisation of the 120-base RNA (*a*, lane 2) 0.5-cm slices were cut from the polyacrylamide gel from positions above (lane 2), coincident with (lane 3) and below (lane 4) the run-off RNA. The slices were crushed and then incubated in hybridisation conditions (see Fig. 2 legend) with Southern strips containing electrophoretically separated fragments of pMB9–1.8-kilobase hybrid plasmid DNA that had been cleaved with *Eco*RI and *Hin*cII (see Fig. 1). These *Eco*RI–*Hin*cII fragments were visualised in lane 1 of *b* by reaction of a Southern strip with ^{32}P-labelled hybrid plasmid DNA (see Fig. 2 legend).

high molecular weight polypeptide. Proteins eluting at the higher ionic strength (0.41 M KCl) included P^{37}, and this polypeptide was present in a stoichiometry of approximately one per RNA polymerase core molecule. As measured by hybridisation to the 770-base pair fragment (Fig. 6*b*) and by the synthesis of the 120-base RNA (Fig. 6*c*), transcription of the 0.4-kilobase gene was strongly correlated with fractions containing P^{37}. Moreover, RNA polymerase in P^{37}-containing fractions exhibited a marked preference for the 0.4-kilobase gene; as shown in the hybridisation experiment of Fig. 2 (lane 4), enzyme in the peak fraction (fraction 36) for P^{37} content copied RNA with high selectivity from the sporulation-controlled sequence and generated less RNA from the vegetative gene or other regions of the hybrid plasmid.

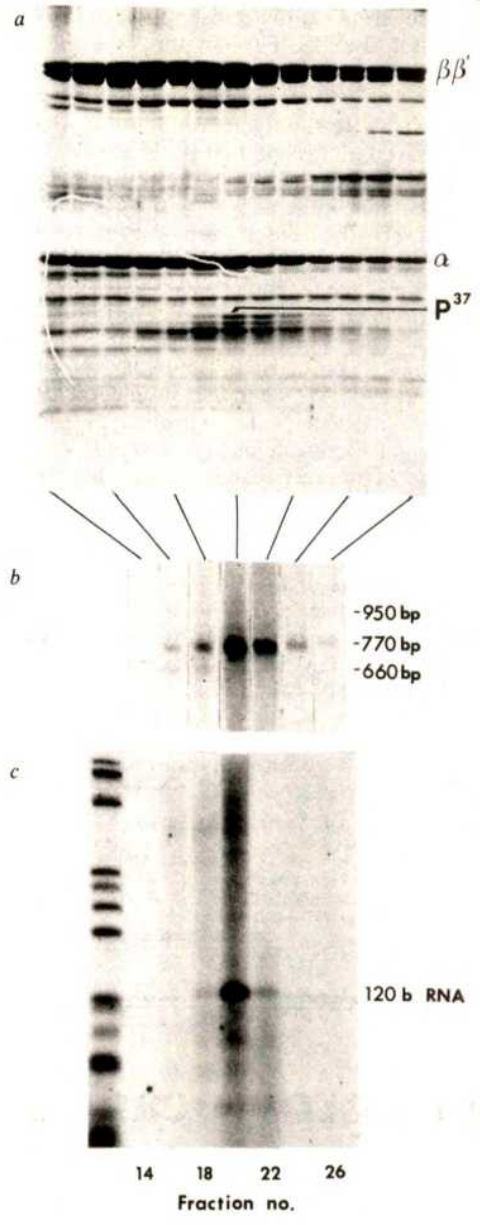


Fig. 5 Gradient elution of RNA polymerase from phosphocellulose. 100 g of *B. subtilis* cells (strain SMY) were collected at the first hour (T_1) of sporulation in Difco sporulation medium[16]. The cells were disrupted by two passages through a French pressure cell (8–10,000 p.s.i.) and RNA polymerase was partially purified by phase partitioning and gel filtration as described by Shorenstein and R.L.[12]. Next, 10 mg of gel-filtration-purified enzyme was applied to a phosphocellulose column (5 × 1.5 cm) and eluted with a 0.2–0.8 M linear gradient of KCl in 100 ml of buffer C(0.05 M Tris, *p*H 8.0, 20% glycerol, 0.3 mM dithiothreitol, 1 mM EDTA). Fractions of 2 ml were collected. *a*, Samples of 100 μl from each fraction were subjected to electrophoresis through a 23-cm-long slab gel containing SDS in a Tris-glycine buffer and a 10–20% gradient of acrylamide[17]. The gel was stained with Coomassie brilliant blue. *b*, RNA was generated *in vitro* from pMB9–4.6-kilobase plasmid DNA in reaction mixtures containing 2.5-μl samples from every other fraction along the gradient and annealed with Southern strips containing *Eco*RI–*Hin*cII fragments of hybrid plasmid DNA. Hybrids were visualised by autoradiography. The panel displays hybridisation in the region of the Southern strips containing *B. subtilis* fragments of 770 and 660 base pairs and a pMB9 fragment of 950 base pairs. Methods for this experiment were described in Fig. 2 legend. The 120-base run-off RNA was synthesised as described in Fig. 4 legend in reaction mixtures containing 2.5-μl samples from every other fraction from the gradient. The products of *in vitro* RNA synthesis are displayed by polyacrylamide gel electrophoresis and autoradiography. MW standards (see Fig. 4) are displayed on the left.

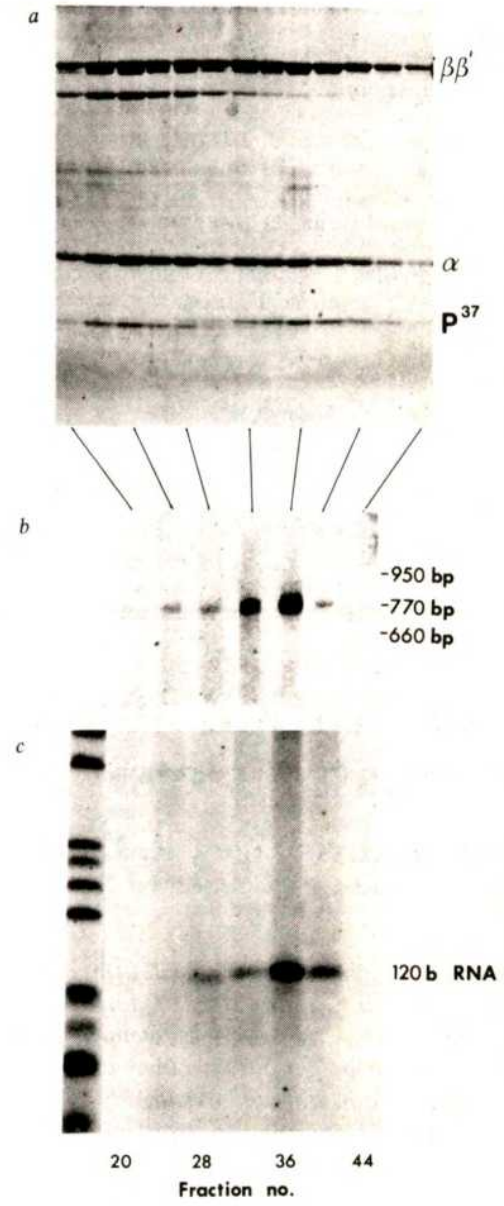


Fig. 6 Gradient elution of RNA polymerase from DNA-cellulose. Phosphocellulose-purified RNA polymerase in fractions 20–22 from the experiment of Fig. 5 were pooled and applied to a calf thymus DNA cellulose column (2 × 1 cm). Enzyme was eluted with a 0.2–0.7 M linear gradient of KCl in 50 ml of buffer C (Fig. 5 legend) and fractions of 1.0 ml were collected. *a*, Displays by SDS gel electrophoresis the pattern of polypeptides in 300-μl samples from every other fraction from the gradient. *b*, Displays the pattern of hybridisation to Southern strips of RNA synthesised *in vitro* by 5-μl samples from every fourth fraction from the gradient. *c*, Displays by polyacrylamide gel electrophoresis the *in vitro* synthesis of 120-base run-off RNA from 5-μl samples from every fourth fraction from the gradient. Procedures for the experiments of *a*, *b* and *c* are as described for the corresponding panels of Fig. 5.

Initiation of spore development

We have isolated a form of RNA polymerase that transcribes a cloned gene[3] (the 0.4-kilobase gene) that is activated at an early stage of sporulation in *B. subtilis*. As evidence of specific transcription, this RNA polymerase copied RNA selectively and asymmetrically from the 0.4-kilobase gene, initiating at a unique site close to the apparent startpoint for RNA synthesis *in vivo*. Nucleotide sequencing should now enable us to define this site precisely.

Purified RNA polymerase capable of transcribing the 0.4-kilobase gene was associated with a polypeptide of MW 37,000, termed P^{37}. P^{37} co-purified with the 0.4-kilobase gene transcribing activity during gradient elution from both phosphocellulose and DNA-cellulose. It therefore seems likely that this species confers on RNA polymerase the ability to recognise and transcribe the 0.4-kilobase gene. This possibility can now be tested directly by reconstructing the enzyme complex from purified P^{37} and core RNA polymerase. If P^{37} is, in fact, a transcriptional determinant, we would suppose that, acting in the apparent absence of σ factor, it is itself a σ-like regulatory subunit of polymerase that directs recognition of the 0.4-kilobase gene promoter. As a precedent, a regulatory subunit of RNA polymerase encoded by *B. subtilis* phase SP01 causes core RNA polymerase to bind at the promoters for phage SP01 'middle' genes[6].

The initiation of sporulation in *B. subtilis* is controlled by at least five or six genetic loci known as the stage 0 genes[1]. Mutations in stage 0 genes *spo0A*, *spo0B*, *spo0E*, *spo0F* and *spo0H* block sporulation at its earliest morphological stage (stage 0) and severely restrict transcription of the 0.4-kilobase gene *in vivo* (ref. 3 and J. F. Ollington, W.G.H. and R.L., in preparation). Based on these and other observations[7-9], we believe that the stage 0 gene products turn on early sporulation genes by modifying RNA polymerase. It is tempting to suppose that P^{37} is involved in this and that P^{37} is itself the product of a stage 0 gene. In recent experiments, however, we find that P^{37} is present in vegetative cells as well as in sporulating bacteria. Possibly, P^{37} directs the transcription of several classes of genes but its involvement in sporulation RNA synthesis is specifically controlled by the stage 0 gene products. This could be investigated by identifying the structural gene for the P^{37} protein.

The isolation from sporulating cells of an RNA polymerase which transcribed a sporulation gene *in vitro* is consistent with suggestions that spore development in general[10] and the 0.4-kilobase gene in particular[3] are controlled, at least in part, by one or more regulatory proteins that bind to RNA polymerase. It will now be of interest to determine whether temporally controlled classes of sporulation genes are transcribed by different forms of RNA polymerase containing distinct regulatory subunits.

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Identification and processing of proglucagon in pancreatic islets

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Immunoprecipitation and tryptic peptide analysis of newly synthesised proteins from rat islets have identified an 18,000 *molecular weight (MW) protein as proglucagon. Conversion of this precursor was kinetically similar to the conversion of proinsulin and resulted in the formation of both pancreatic glucagon and a* 10,000*-MW protein lacking this hormonal sequence.*

AFTER the detection of proinsulin in 1967 (ref. 1) the search for a biosynthetic precursor of glucagon became the subject of considerable investigation. Several investigators have found larger immunoreactive forms of glucagon in extracts of pancreas[2,3], of gastric and intestinal mucosa[4,5], of salivary glands[6], and in plasma[7]. Estimates of the molecular weights of these various forms range from 4,500 to 70,000, but only a few forms have been characterised. Thus, we isolated a peptide which carried a C-terminal extension of eight amino acids on the sequence of pancreatic glucagon[8]. Because of its small size (MW 4,500) and because of the identification of low amounts of larger forms, this peptide seemed to represent a fragment or intermediate cleavage form of proglucagon. More recently, a larger form has been isolated from porcine intestine and has been partially sequenced. This peptide, glicentin[9], consists of 100 amino acid residues and has a molecular weight of 12,000. The glucagon moiety in this molecule is also extended at the C-terminus by a sequence of eight amino acids very similar to that of the 4,500-MW proglucagon fragment. More recently, 12,000 and 8,000-MW glucagon-containing peptides of intestine have been found to have identical counterparts in pancreatic tissue[10].

Although the above findings have not established the precursor nature of any of the larger forms of glucagon, biosynthetic studies in isolated fish islets have indicated that proglucagon has a molecular weight of 12,000 (refs 11, 12). In the present study we have used the high resolving power of SDS–polyacrylamide gel-electrophoresis and the sensitivity of fluorographic methods to examine the biosynthesis of glucagon in pancreatic islets of

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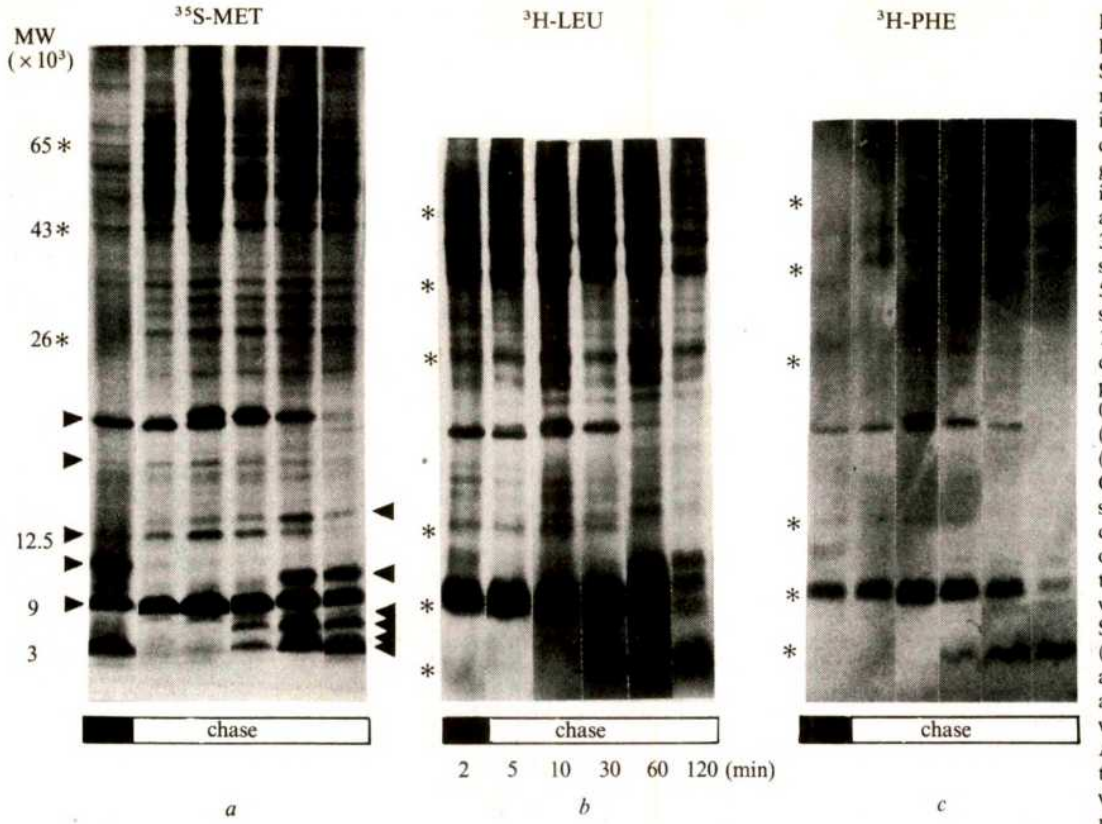


Fig. 1 Pulse-chase labelling of islet proteins. Samples of 100 isolated rat islets[30] were incubated in 50 μl Hanks' buffer containing 2.5 mM glucose and 50 μCi of the indicated radioactive amino acid for 2 min at 37 °C. Pulse-labelling was stopped by the addition of 5 ml Hanks' buffer supplemented with 10^{-3} M of the respective cold amino acid. Chase periods, in minutes, were 0 (lane 1), 3 (lane 2), 8 (lane 3), 28 (lane 4), 58 (lane 5) and 118 (lane 6). Chase-incubation was stopped by chilling, centrifugation and boiling of islets in 12.5 μl denaturing solution[20]. Samples were electrophoresed on SDS-polyacrylamide gels (0.1% SDS/16% acrylamide/0.25% bisacrylamide[16,31]) and dried gels were fluorographed[32,33]. Asterisks show the position of marker proteins whose sizes are indicated numerically on the left. Arrowheads to the left of a point (from top to bottom) to proglucagon (MW 18,000), an unidentified prohormone-like protein (MW 16,000), prosomatostatin (MW 12,500) (unpublished), preproinsulin[16], and proinsulin. Arrowheads at the right point to conversion products.

the rat. Immunoprecipitation, tryptic peptide analysis and kinetic studies of formation and processing have all identified an 18,000-MW peptide as pancreatic proglucagon.

Prohormone-like proteins in pancreatic islets

To identify proteins having prohormone-like kinetics of formation and processing[13–15], pulse-chase experiments were carried out with isolated rat islets using various radioactive amino acids of high specific activity. The slab gel electrophoretic system was optimised by adjusting the concentrations of acrylamide and bisacrylamide[16] for resolution of proteins in the molecular weight range of 10,000–30,000, a size range in which new prohormones might be expected to occur[17]. In the pulse-chase experiments shown in Fig. 1 proinsulin was the predominant labelled band and was used as an internal standard for the detection of other proteolytically processed proteins. The most conspicuous protein which exhibited prohormone-like behaviour was a relatively highly labelled band of MW about 18,000, which appeared immediately after a pulse of 2 min. However, unlike proinsulin, this 18,000-MW protein underwent a small post-translational increase in its molecular size. It later disappeared after a chase period of 30–120 min. This period for post-translational processing is consistent with prohormone conversion, as indicated by the fading of the labelled proinsulin band and the appearance of smaller insulin-like conversion products (Fig. 1). These results suggested that the 18,000-MW band might represent a precursor to one of the other major islet hormones such as glucagon or somatostatin. Faintly labelled proteins showing similar labelling kinetics, though without early post-translational enlargement, could also be found consistently with MW 12,500 and, in the case of experiments *a* and *b* of Fig. 1, with 16,000 MW.

Proteins appearing later in the course of a chase-period are likely to represent intermediates or endproducts of the conversion process. Thus a 13,000-MW protein and one of MW 10,000 are, on the basis of their relative abundance, both likely to be derived from the 18,000-MW precursor (Fig. 1). Furthermore, up to four labelled peptides smaller than proinsulin were resolved as indicated by arrowheads in Fig. 1*a*. These proteins were included, together with the above-mentioned candidate prohormones, in the surveys for proteins involved in the biosynthesis of glucagon described below. The 12,000-MW protein was identified as the prohormonal form of somatostatin (unpublished), whereas the 16,000-MW band has not yet been related to any known islet hormone.

Identification of glucagon-related proteins

For the identification of proglucagon and its conversion products islets were pulse-labelled for 2 h with ^{35}S-methionine. Islet lysates were electrophoresed and fluorographed as described in Fig. 1 legend and prohormones and conversion products were eluted from the dried gels for immunoprecipitation. Two types of glucagon-antisera were used for this study: the first is a tissue nonspecific antiserum (designated NS) known to be directed toward the central and N-terminal regions of glucagon and known to be reactive with larger forms of glucagon; the second is an antiserum specific for pancreatic glucagon (designated CTS, that is C-terminus-specific) which recognises the amino acid sequence at positions 24–29 of the glucagon molecule but which does not react with forms carrying a C-terminal extension in the radioimmunoassay[10]. Such an extension has been reported to be present in a proglucagon fragment isolated from commercial glucagon preparations[8], in glicentin[9], and in 12,000 and 8,000-MW glucagon-containing peptides of both pancreas and intestine[10]. It can be removed by combined treatment with trypsin and carboxypeptidase B. The authentic C-terminal tryptic fragment of glucagon cleaved out of a larger molecule by this enzymatic treatment would also be recognised by this specific antibody[10].

The data in Fig. 2 shows that the two proteins of MW of 18,000 are the only candidate prohormones which exhibited significant reactivity with the glucagon antisera. In addition, a

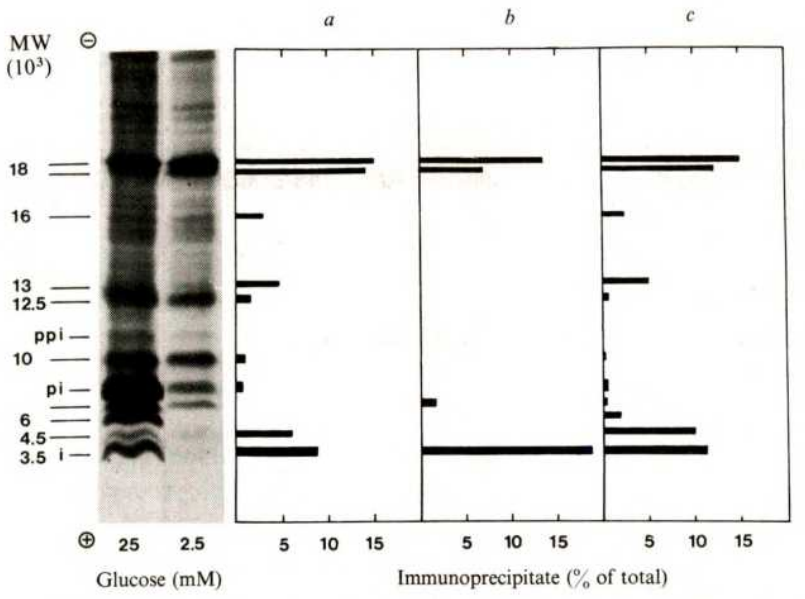


Fig. 2 Immunoprecipitation of newly synthesised islet proteins with various glucagon antisera. Islets (1,200) were incubated in Hanks' buffer containing 2.5 mM glucose and 600 μCi ^{35}S-methionine (Amersham) for 2 h at 37 °C. Islets were dissolved in 150 μl denaturing solution with boiling, the sample then being distributed to 12 slots of an SDS-polyacrylamide-gel[7,16] slab for electrophoresis and subsequent fluorography. The lower molecular weight range of one lane of the resulting fluorograph is shown at the left side in comparison to a sample incubated at 25 mM glucose. Protein bands as indicated at the margin (see also Fig. 1*a*) were eluted from excised gel pieces, either by shaking for 48 h at 37 °C in 2 × 2 ml of 0.01 M NH_4HCO_3 (proteins < 6,000-MW), or by shaking in 2 × 2 ml 25 mM Tris/200 mM glycine/0.1% SDS (proteins > 6,000-MW). Bovine serum albumin (BSA) (200 μg) was added to all samples as carrier. SDS was removed by dialysis against the SDS-free buffer for 72 h. Concentration of the samples was achieved by precipitation in 10% TCA or by lyophilisation (fractions < 6,000-MW). All fractions were finally dissolved in 0.7 ml NH_4HCO_3 (0.1 M). For immunoprecipitation 50-μl samples were incubated after addition of 150 μl of 0.1 M Tris-HCl, *p*H 7.6, 0.05 M NaCl, 0.01 M EDTA, 0.1% BSA with 1 μl glucagon antiserum NS (*a*) or CTS (*b*). Samples for set *c* were pretreated at analogous amounts with 2 μg tosylphenylalanine chloromethyl ketone-treated trypsin (Worthington) for 12 h at 37 °C in 0.5 ml 0.1 M NH_4HCO_3/2 mM $CaCl_2$. Reaction was stopped by addition of 5 μg phenylmethylsulphonyl fluoride (in absolute ethanol) and 10 μg carboxypeptidase B (Boehringer) was added for a further incubation period of 12 h at 37 °C. Reaction was finally stopped by boiling, samples were lyophilised and prepared for immunoprecipitation with antibody CTS as described above. Controls to which 10 μg of glucagon were added before the addition of the antiserum were always included. All samples were run as duplicates. After 24 h incubation the appropriate anti-IgG serum was added for double-antibody precipitation. Precipitates were washed in excess buffer containing 1% Triton X-100. Pellets were finally dissolved in 0.2 ml 3 M acetic acid and counted in 10 ml Aquasol (New England Nuclear). Data represent means of two different experiments (except for the 13,000 and 16,000-MW proteins). ppi, Preproinsulin; pi, proinsulin; i, insulin.

low molecular weight fraction co-migrating with iodinated glucagon (MW 3,500) and a somewhat slower migrating peptide (MW 4,500) exhibited immunoreactivity; to a lesser extent the 13,000-MW protein was also precipitated with antiglucagon serum. Whereas the 3,500-MW fraction reacted with the C-terminus specific antibody without enzymatic pretreatment (Fig. 2*b*) the 4,500 and 13,000-MW proteins showed such reactivity only after trypsin plus carboxypeptidase B-treatment (Fig. 2*b* versus *c*) and may thus carry a C-terminal extension on their glucagon moieties. On the basis of its estimated size and its immunological behaviour, the 3,500-MW peptide is regarded as intact glucagon, whereas the 4,500-MW glucagon-like component probably represents the proglucagon fragment described earlier[8]. The 13,000-MW protein, which might be contaminated by the adjacent band (prosomatostatin), may be an intermediate in the conversion process. Immunoprecipitation of the intact 18,000-MW peptides by the C-terminus-specific antibody was not unexpected, inasmuch as this antiserum will react with C-extended forms in conditions of antibody excess, presumably due to the presence of small amounts of non-discriminating antibodies (unpublished results).

To clarify the nature of the 18,000-MW component the labelled 18,000-MW protein was subjected to tryptic cleavage and two-dimensional analysis. The presence of the labelled peptides of glucagon in the tryptic digest would confirm the identification of this protein as proglucagon. Analysis of the C-terminal tryptic fragment(s) before and after treatment with carboxypeptidase B would allow the detection of an additional C-terminal basic amino acid, indicating the presence of an extension at this site. The 10,000-MW fragment which, as shown in Fig. 2, was found to be unreactive with the glucagon antisera, was included in this peptide analysis. The relevant peptide maps are presented in Fig. 3. It is evident from these maps that the 18,000-MW protein contained the N-terminal tryptic peptide (labelled with ^{3}H-phenylalanine, Fig. 3*c*, left panel) as well as the group of four peptides related to the C-terminal tryptic fragment of glucagon. This latter group of peptides, however, migrated to the positions of the respective ninhydrin-stained peptides of the glucagon carrier only if the tryptic digestion products were treated with carboxypeptidase B (Fig. 3*b*, left panel). Trypsin-treatment alone did not result in the formation of these ^{35}S-methionine-(Fig. 3*a*) or ^{3}H-phenylalanine-(Fig. 3*c*) labelled peptides of the glucagon-C-terminus, thus supporting the assumption that the C-terminus of the glucagon-moiety in this prohormone is extended by an additional sequence.

In addition, release of the N-terminal tryptic peptide of glucagon during digestion of the 18,000-MW protein with trypsin (Fig. 3*c*, left panel) shows that the glucagon sequence in this precursor is linked to its N-terminal extension by at least one basic residue.

Comparison of the maps of the 18,000-MW protein with those of the 10,000-MW protein (Fig. 3, left versus right panels, respectively) shows that these two proteins have many tryptic peptides in common, but that digests of the 10,000-MW fragment lack the corresponding glucagon-related peptides. These results agree with the immunoprecipitation data of Fig. 2, that is, the glucagon-related sequence was removed during the conversion of proglucagon to the 10,000-MW fragment.

Conversion of proglucagon

These results suggest that the breakdown of the 18,000-MW proglucagon to its 10,000-MW fragment should be accompanied either by the generation of a glucagon-containing fragment or by the formation of labelled glucagon itself. For a

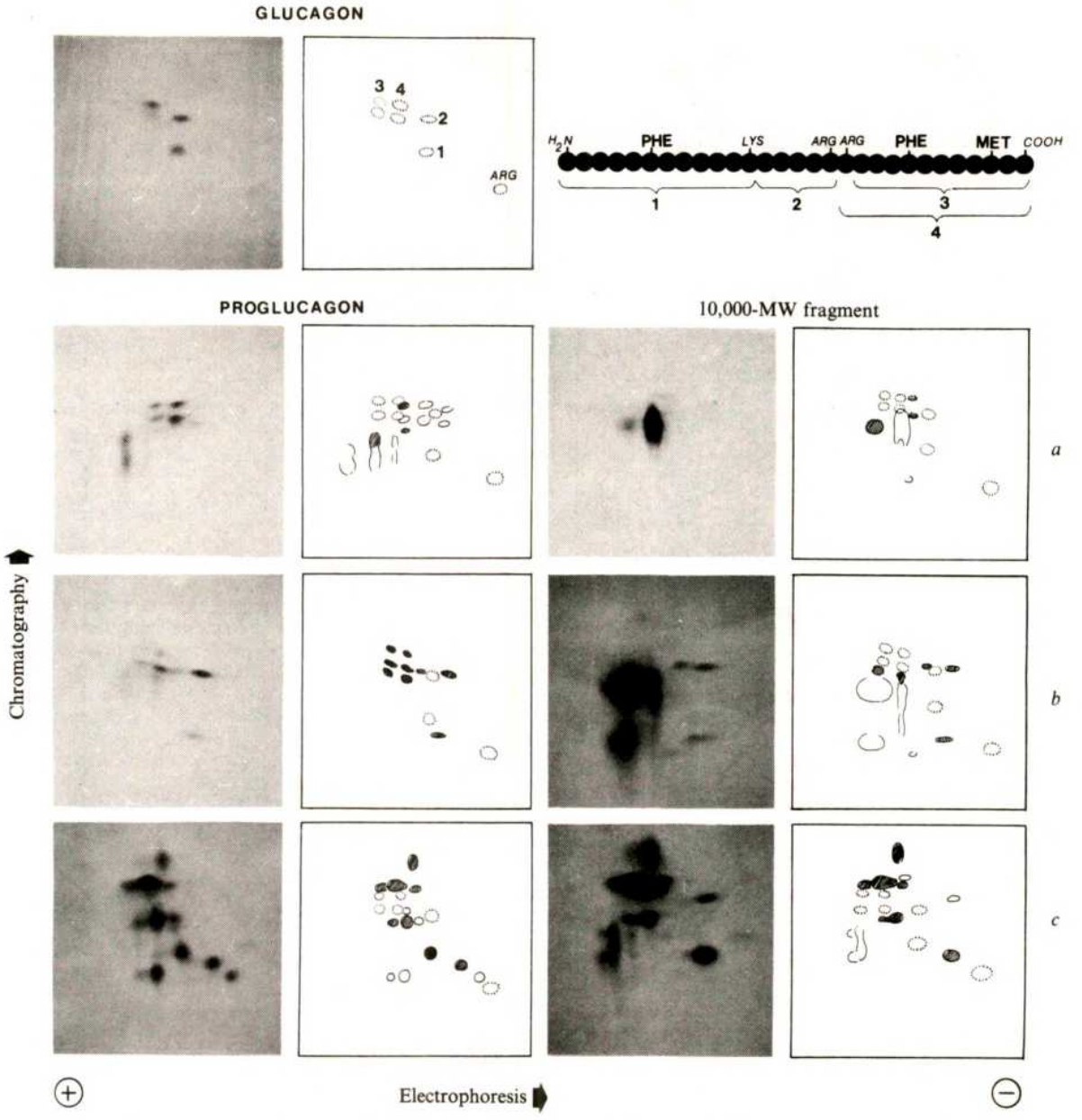


Fig. 3 Tryptic peptide analysis of proglucagon and its 10,000-MW fragment. 18,000 and 10,000-MW proteins, labelled for 2 h and eluted from the fluorographed electrophoresis gel, were treated with trypsin (*a* and *c*) or trypsin plus carboxypeptidase B (*b*) as described in Fig. 2 legend. The proteolytic digests were lyophilised, the tryptic peptides of 50 μg glucagon were added, and samples were electrophoresed on 20×20 cm cellulose-thin layer plates in 30% formic acid in the first and chromatographed in *n*-butanol/pyridine/acetic acid/water (60:40:12:48, v/v) in the second dimension. Plates were coated in PPO[34] and fluorographed. After exposure, plates were stained with ninhydrin reagent for detection of the peptides of the glucagon carrier. The figure shows at the upper left the pattern of the tryptic fragments of glucagon. Set *a* shows the fluorographed peptide maps of the tryptic digests of the respective ^{35}S-methionine labelled proteins, set *b* the trypsin + carboxypeptidase B digests of the same proteins, whereas under *c* the tryptic peptides of the ^{3}H-phenylalanine-labelled proteins are shown. Diagrams at the right side of the respective maps: dotted spots, ninhydrin-stained glucagon peptides (not visible on the fluorographs); hatched spots, peptides that are common to both proteins (proglucagon and 10,000-MW fragment); black spots, labelled spots on the fluorograph at the position of ninhydrin-stained tryptic glucagon fragment(s).

comparison of the kinetics of formation of proglucagon and its fragments, islets were 'pulse'- and 'pulse-chase'-labelled. The indicated bands were excised from the fluorographed gels and the radioactivity was quantitated by liquid scintillation spectrometry. Due to the similarity of the peptide patterns of proglucagon and the 10,000-MW fragment (Fig. 3) the presence of major contaminants in these bands is unlikely. Because of contaminating labelled peptides in the 3,500-MW band, labelled glucagon was quantitated after immunoprecipitation of the eluted material.

The data of the pulse-chase experiment illustrated in Fig. 4*a* show that the prohormone was converted into its slightly larger form within 10–30 min, and then began to decrease as label appeared in the 10,000-MW component. Immunoprecipitable, labelled glucagon was not detected in this experiment, however. With prolonged pulse-labelling (Fig. 4*b*) the expected saturation of incorporation of labelled amino acids into the two forms of proglucagon occurred within the first 60 min. Formation of the 10,000-MW protein began after a delay of 30–60 min and then continued almost linearly for the period up to 3 h. Labelled glucagon was detected in the eluate of the 3,500-MW band (Fig. 4*b* bottom panel), but its formation seemed to be somewhat slower than that of the 10,000-MW fragment. The 13,000-MW protein which showed some glucagon-like immunoreactivity (Fig. 2) was also included in these analyses. However, its kinetic behaviour did not clearly indicate whether this protein is a transient intermediate or a side-product of the conversion process (see discussion).

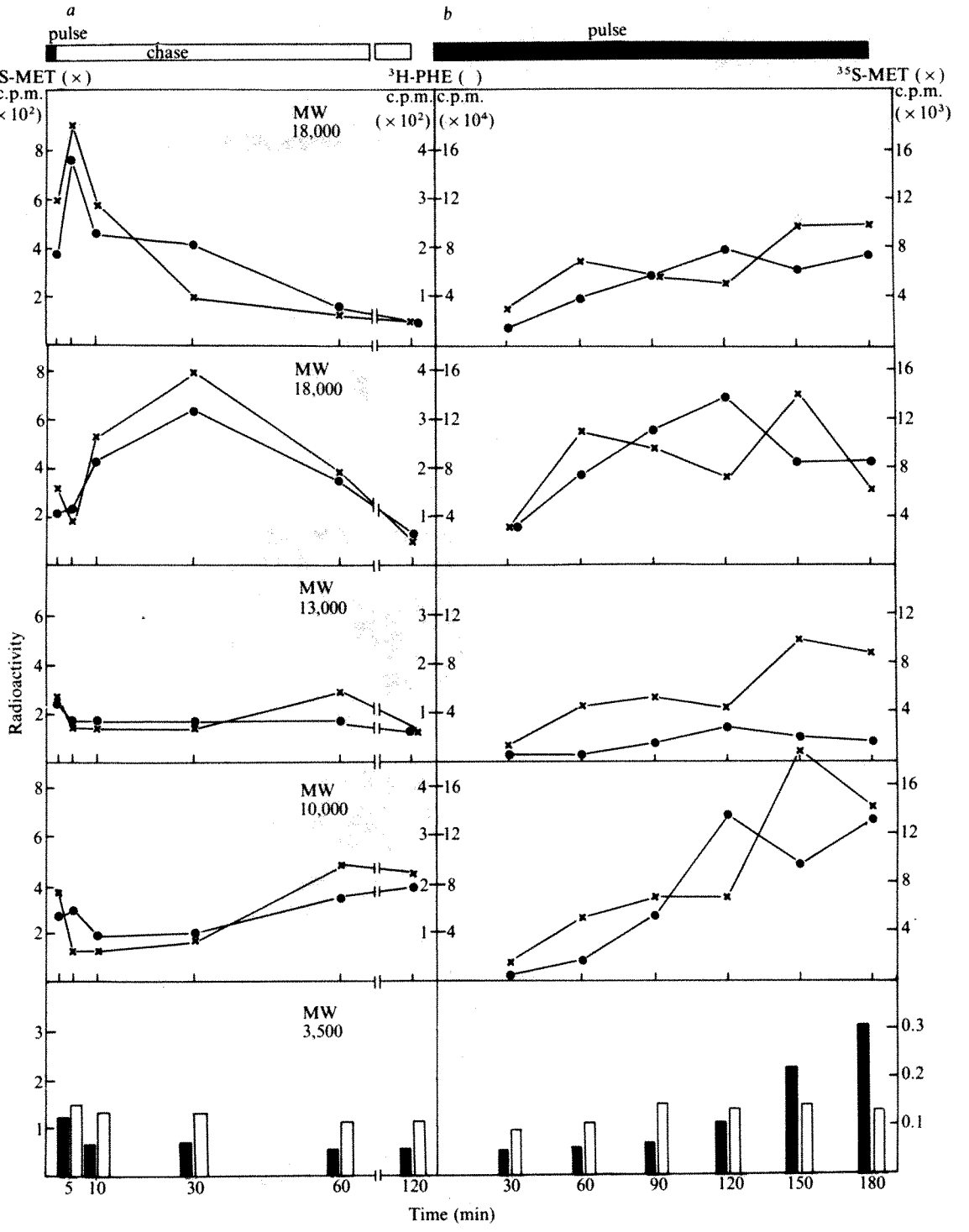


Fig. 4 Kinetics of formation and conversion of glucagon-related proteins. *a*, The pulse-chase experiments are those shown in Fig. 1*a* and *c* (see legend for details). *b*, For prolonged pulse-labelling samples of 100 islets were incubated for the indicated period and islet lysates were treated for electrophoresis as described before. The respective bands were excised from fluorographed gels, dissolved in 0.2 ml 30% H_2O_2 and counted in 10 ml Aquasol. The 3,500-MW protein was eluted from the respective band and immunoprecipitated as described in Fig. 2 legend. All data represent means of two determinations. ■, Immunoprecipitates; □, controls.

Comparison of the scales in Fig. 4 shows that the accumulation of label in the 10,000-MW fragment and in glucagon, respectively, differ by a factor of about 50. This discrepancy may be due to the presence of more than one methionine residue in the 10,000-MW protein, incomplete immunoprecipitation of glucagon or the accumulation of other glucagon-containing intermediates similar to the 4,500-MW peptide (Fig. 2) which were not included in these calculations. Furthermore, considerable losses of glucagon from the gel may have occurred during treatment for fluorography. Such losses have been found to be negligible for proteins in the range larger than 8,000–9,000 MW.

Quantitative aspects of prohormone synthesis in pancreatic islets

Pancreatic islets are composed of at least four different cell types each of which produces at least one known hormone, namely B cells (insulin), A cells (glucagon), D cells (somatostatin) and a fourth cell type which produces pancreatic polypeptide. The relative numbers of these cell types have been determined in

Table 1 Comparison of cellular composition and prohormone biosynthesis in pancreatic rat islets

Cellular composition			Prohormone synthesis			
					^{14}C-AA incorporation(c.p.m.)*	
Cell type	Hormone	% Of islet volume	Prohormone	MW	*a*	*b*
B cell	Insulin	56	Proinsulin	9,000	4,561 (66)	15,520 (87)
A cell	Glucagon	16	Proglucagon	18,000	1,766 (26)	1,566 (8)
D cell	Somatostatin	5	Prosomatostatin	12,500	200 (3)	287 (2)
PP cell	Pancreatic polypeptide	5	Pancreatic propolypeptide	—		
?	?		Unidentified prohormone	16,000	307 (5)	410 (3)

* Samples of 100 islets were preincubated for 2 h at 37 °C at 2.5 (*a*) or 25 (*b*) mM glucose. 20 μCi of a ^{14}C-labelled amino acid mixture (Amersham) was then added and incubation was continued for 20 min. Islets were washed, lysed and electrophoresed as described in the Fig. 1 legend. The respective bands were excised from the fluorographed gels and scintillation-counted (see Fig. 4 legend). Data represent means of four determinations. Relative prohormone synthesis is given in parentheses.

islets of several species, including rats[18,19]. Table 1 gives a synopsis of these data in comparison with the individual rates of biosynthesis of the prohormones identified thus far. As glucose has been reported not only to have a stimulatory effect on insulin synthesis but also an inhibitory effect on the synthesis of glucagon[20,21], prohormone synthesis was studied after preincubation of islets for 2 h at low (2.5 mM) and high (25 mM) glucose concentrations. To compensate for differences in amino acid composition, incorporation was measured with a mixture of ^{14}C-labelled amino acids. Although this experiment is based on some assumptions, the biosynthetic data compiled in Table 1 (right panel) correlate remarkably well with the proportion of the various cell types in rat islets (Table 1, left panel). Whereas proinsulin-synthesis was stimulated fourfold by preincubation at high glucose concentrations the formation of the other prohormones remained almost unaltered in agreement with our own previous findings (Fig. 2, ref. 11).

Discussion

On the basis of its kinetics of formation and processing, immunological analysis and tryptic peptide mapping, the 18,000-MW protein of rat pancreatic islets must be regarded as proglucagon. Final proof of this conclusion may come from the identification of the primary translation product of the respective mRNA as preproglucagon, the putative presecretory form which, if typical, should have a molecular weight of about 21,000. Preliminary evidence for such a translation production has been obtained by translation of RNA extracted from a human pancreatic endocrine tumour[35]. Although preproinsulin was detectable after pulse labelling of intact islets (Fig. 1, ref. 11), a similarly short-lived protein of the presumed size of preproglucagon was not detectable in the experiments reported here. The post translational modification of the 18,000-MW protein which results in an increase in its molecular size has not yet been identified. Glycosylation does not seem to be involved as judged from the very low incorporation of various labelled sugars, the lack of binding to various lectins, and the lack of any inhibition of its biosynthesis by tunicamcycin, a known inhibitor of protein glycosylation[22].

With regard to the position of the glucagon sequence within the prohormone, we can conclude that the hormonal sequence is not located at the C-terminus of the precursor. The arrangement

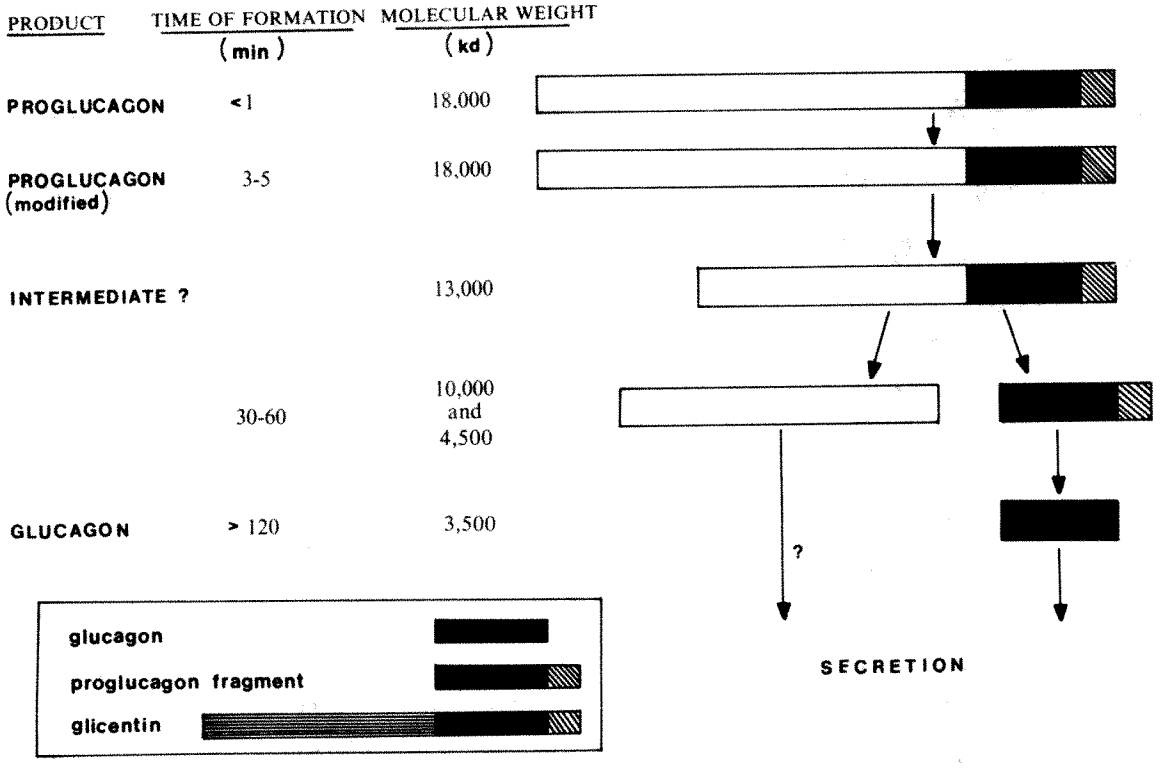


Fig. 5 Proposed scheme for the conversion of proglucagon to glucagon in pancreatic rat islets.

proposed in the scheme shown in Fig. 5 was chosen by analogy to the position of glucagon in glicentin[9], which has been demonstrated by immunohistochemistry to be present also in the rat pancreatic A cell[23]. Immunoprecipitation of the proteins listed in Fig. 2 with antiserum R-64, which is directed towards the non-glucagon portion of porcine glicentin[24], however, did not provide evidence for such immunoreactivity in proglucagon or any of its conversion products. This lack of reactivity may most simply be interpreted to indicate that larger forms of glicentin are poorly reactive with this antiserum or that some damage to the antigenic site has occurred during electrophoresis and elution of the peptides. Alternatively, it is possible that multiple genes for proglucagon-like peptides occur in the rat as is known to be the case with the insulin gene[12], and that glicentin is a minor component of the pancreatic alpha cell[10].

The sequential conversion of proglucagon can only be inferred to correspond to the scheme shown in Fig. 5. Our data support the conclusion that, in addition to glucagon, the 10,000-MW fragment is an end product of proglucagon conversion and might therefore represent a so far unidentified hormonal entity. It is not yet known whether this fragment is secreted along with glucagon. The 13,000 and 4,500-MW may represent intermediates in the conversion process, but they could also represent side-products of this process. Similar explanations may apply to several larger forms detected in glucagon-producing tumours[25], or in plasma in normal and various clinical conditions[7,25]. In any case, such intermediates or side products may have escaped detection during the 2-h period used in our biosynthetic experiments, whereas the short-lived prohormone might be overlooked during immunochemical studies on tissue extracts.

This prohormone, about five times the size of glucagon, might well contain additional hormonal sequences. Immunohistochemical techniques have suggested that additional hormones, including gastric inhibitory polypeptide[26,27], cholecystokinin[28] and even β-endorphin[29], may be present in pancreatic A cells. Our identification of a distinct protein as the prohormone of glucagon represents the first step in its further characterisation, which will ultimately answer the question of the possible presence of additional hormonal sequences, the question of the nature of the post translational modification as well as that on the mode of prohormone conversion.

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The heavy chain of human histocompatibility antigen HLA-B7 contains an immunoglobulin-like region

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The 88-residue fragment (ac-2) containing the second disulphide loop from HLA-B7 heavy chain is shown to have statistically significant homology with Ig constant domains, including both matches at invariant positions and conservative substitutions of structurally important residues. Thus, both the light chain (β_2m) and a segment of the heavy chain of HLA antigens may be structurally and evolutionarily related to immunoglobulins.

AMONG the products encoded by the major histocompatibility complex (MHC)[1–3] are the highly polymorphic antigens coded for by the H-2K and H-2D loci in the mouse and the HLA-A, HLA-B and HLA-C loci in man. Besides being target antigens during graft rejection, these proteins are important in the recognition of both virally infected[4,5] and chemically modified[6,7] syngeneic cells by cytotoxic T lymphocytes. These membrane antigens consist of a glycosylated heavy chain of MW 44,000 and a light chain of MW 12,000[8–11]. The larger chain is variant and is thought to be coded for by the MHC. The smaller chain is invariant and has been identified as β_2-microglobulin (β_2-m)

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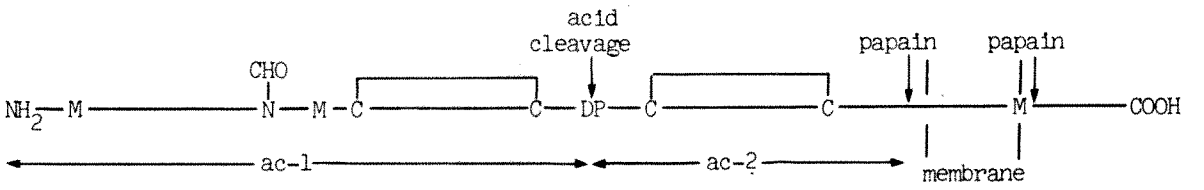


Fig. 1 Localisation of the ac-2 fragment within the HLA-B7 heavy chain. Papain-solubilised HLA-B7 heavy chain (MW 34,000) was purified from the human lymphoblastoid cell line JY as described elsewhere[21]. The ac-2 fragment was obtained by partial acid hydrolysis in 70% formic acid at 37 °C (ref. 19) followed by gel filtration. ac, acid cleavage fragment; CHO, carbohydrate moiety; M, Met; N, Asn; C, Cys; D, Asp; P, Pro.

which is not coded for by the MHC. An interesting feature of β_2-m is its primary structural homology with immunoglobulin (Ig) constant domains[12,13]. As the constituent chains of many multi-subunit proteins are structurally homologous (for example, haemoglobins, immunoglobulins and many multi-subunit enzymes), it was tempting to speculate that the heavy chain of HLA-A and -B would contain segments resembling β_2m and therefore Ig-like domains. Such homology would also further support the notion of common evolutionary origins for the immunoglobulin and histocompatibility systems[14–16]. Early reports of HLA-A and -B similarity to Ig were based on indirect evidence such as overall subunit structure[17,18], susceptibility to limited papainolysis[18] and the arrangement[19] and size[20] of the intrachain disulphide loops. When preliminary data on the primary structure of the HLA-A and -B heavy chain became available, several authors reported sequence homologies with β_2m and various Ig domains[19,21–24]. However, all of these preliminary reports were based on short segments of the HLA-A and -B heavy chain.

In this article, the complete amino acid sequence of the 88-residue fragment (ac-2) containing the second disulphide loop of the HLA-B7 heavy chain is shown to display statistically significant homologies to Ig constant domains and β_2m. This homology is discussed in terms of its possible relation to structure and evolution.

Comparison of the ac-2 fragment of HLA-B7 and Ig constant domains

The ac-2 fragment comprises the COOH-terminal region of papain-solubilised HLA-B7 heavy chain (see Fig. 1). Sequence determination of papain-solubilised HLA-B7 heavy chain, including ac-2, is described in detail elsewhere[25,26]. The sequence of the ac-2 fragment was aligned with human Ig constant domains and β_2m[27–32] (Fig. 2; see legend for detailed procedure). These latter proteins are members of a single superfamily[32].

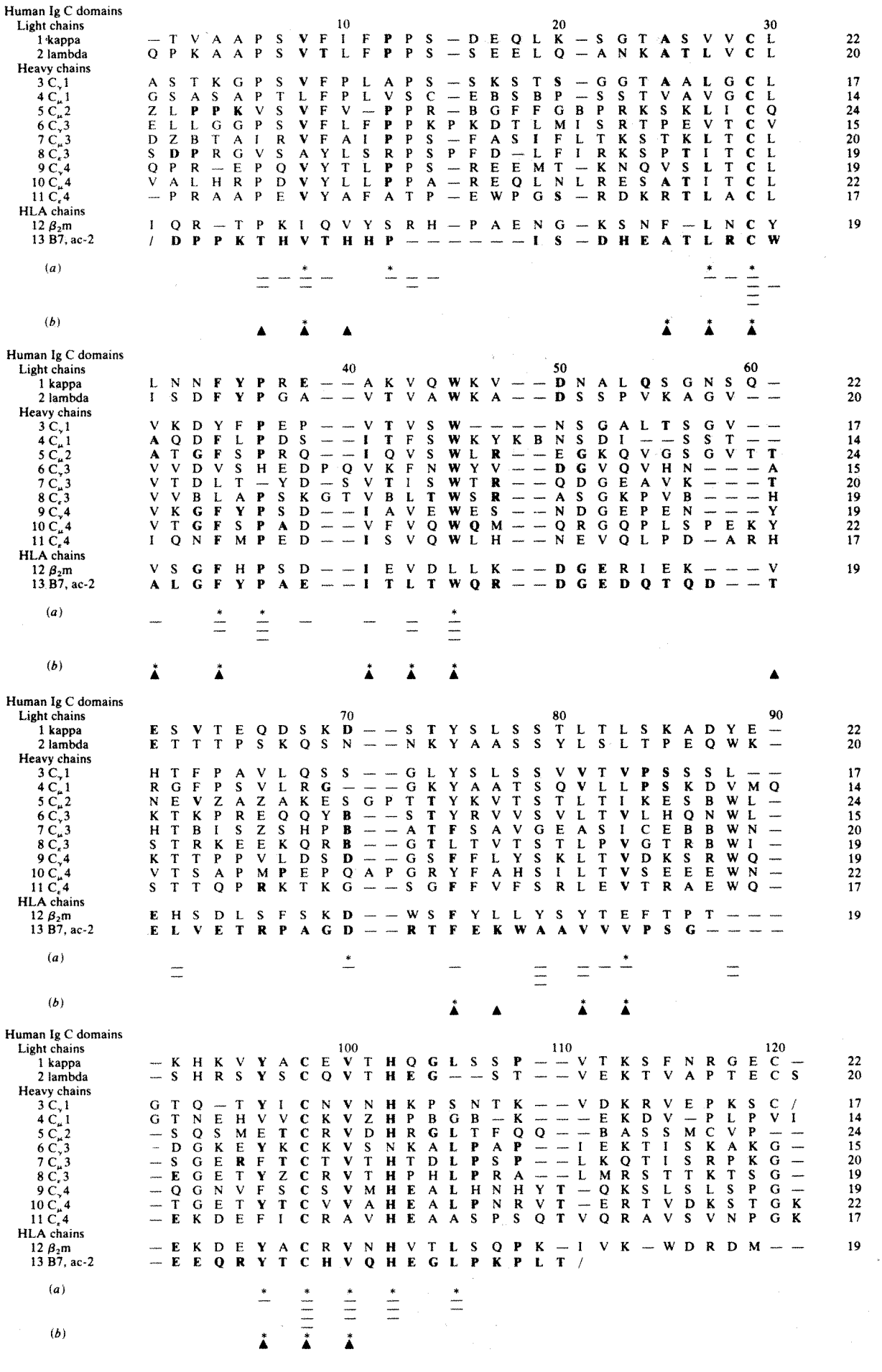
The significance of the sequence homology between ac-2 and Ig constant domains becomes more apparent by considering the positions involved: many of those are highly conserved among different Ig-like domains. The two cysteines (29 and 98) appear in all sequences of Fig. 2 and actually in all Ig domains, including the variable ones. Trp 45 is also very prominent, appearing in all Ig constant and variable domains. In the three-dimensional structure they are known to occupy a position at the centre of the domain, near the internal disulphide bridge, and probably have a role in chain folding (ref. 33 and refs cited therein). Notably, β_2m does not have this tryptophan, which is replaced by Leu 45. Val 100 and His 102 appear in all but one, Pro 36 appears in all but two and Val 8 appears in all but three sequences in Fig. 2. Of seven residues marked as particularly conserved (that is, they

Table 1 Difference matrix

	C_L	C_H1	C_H2	C_H3	C_H4	β_2m	ac-2	
C_λ	62	76	78	77	75	82	78	C_L
$C_\lambda1$	75	65	82	82	80	88	86	C_H1
$C_\mu1$	82	79	65					
$C_\mu2$	76	79	79	85	78	79	85	79	C_H2
$C_\lambda3$	73	76	77	85	77				
$C_\mu3$	80	78	81	85	78	73	70	77	86	83	C_H3
$C_\varepsilon3$	79	77	79	86	80	67	70				
$C_\lambda4$	75	72	73	82	79	76	76	76					
$C_\mu4$	78	72	76	84	78	72	73	70	64	68	81	81	C_H4
$C_\varepsilon4$	80	74	81	86	81	82	86	79	68	73				
β_2m	80	85	87	90	85	85	86	88	76	86	82		77	β_2m
ac-2	78	79	82	89	79	87	80	82	79	81	83	77		
avg	76.5	75.1	76.9	83.1	79.6	77.5	78.8	77.8	74.7	75.6	79.5	83.9	81.3	
	C_κ	C_λ	$C_\gamma1$	$C_\mu1$	$C_\mu2$	$C_\gamma3$	$C_\mu3$	$C_\varepsilon3$	$C_\gamma4$	$C_\mu4$	$C_\varepsilon4$	β_2m	ac-2	

The difference matrix[27] derived from the alignment of Fig. 2 is shown in the section below the diagonal line. Numbers represent per cent non-identity between pairs of sequences (including matches between a gap and any residue). All values except those involving ac-2 are reproduced from ref. 32. The numbers for ac-2 are calculated as upper limits, assuming that no identities occur beyond position 110. The numbers above the diagonal line are obtained by averaging the values of corresponding domains from different Ig chain classes. The numbers in the lowest row are averages of difference values between a particular sequence and all others. Values for a sequence with itself are excluded from all calculations.

Fig. 2


Human Ig C domains
Light chains
1 kappa
2 lambda
Heavy chains
3 $C_\gamma 1$
4 $C_\mu 1$
5 $C_\mu 2$
6 $C_\gamma 3$
7 $C_\mu 3$
8 $C_\varepsilon 3$
9 $C_\gamma 4$
10 $C_\mu 4$
11 $C_\varepsilon 4$
HLA chains
12 β_2m
13 B7, ac-2
(a)
(b)
10
20
30
40
50
60
70
80
90
100
110
120

appear in at least 80% of the Ig constant domains shown in Fig. 2), six also appear in ac-2 (Cys 29, Pro 36, Trp 45, Cys 98, Val 100 and His 102). In all, there are 30 positions in the Ig constant domain at which one residue appears in at least 50% of the sequences, the ac-2 fragment matches 14 of these residues. All of these residues are marked in Fig. 2.

Another interesting feature of the homology between ac-2 and Ig domains is revealed when the homologous residues are related to the observations of Beale and Feinstein[33] concerning the role of side chains in domain folding. These authors aligned residues occupying analogous positions in the crystallographic structures[34–36]. The resulting alignment for Ig domains and β_2m resembles very much that of Fig. 2, suggesting that sequence homology data for a new member may contain information on its tertiary structure. From their comparison several highly conserved features of Ig domain sequences and relationships to domain folding were identified. Of particular interest is that several of these features can be found in the primary structure of the ac-2 fragment of HLA-B7. Thus a dominant feature of Ig constant domains is that at some positions, substitutions are restricted within a group of hydrophobic amino acids (Phe, Ala, Tyr, Pro, Met, Val, Leu and Ile). The side chains of some of these residues are found to fill the internal space within the domain. Of 19 such residues in Ig domains, 15 are found in ac-2 (see Fig. 2). In Ig, some of these conserved residues form three hydrophobic clusters[33] which may be important in the nucleation of Ig chain folding. Most of the residues participating in the formation of such clusters appear in ac-2. These include all three residues in the first cluster (Val 8, Ala 31 and Val 100), three out of four in the second (Phe 34, Ile 41 and His 102) and two out of three in the third (Leu 27 and Val 81). Beale and Feinstein[33] further noted that many of these conserved positions correspond to the stretches of alternating hydrophobic residues characteristic of the β-sheet structure in Ig domains. Four such stretches seem to appear in ac-2, at positions 25–34, 41–45, 75–83 and 96–100. These features are illustrated in Fig. 3, depicting the human C_λ folding in relation to the ac-2-Ig homology. Of the five residues conserved in the C_λ domain (Fig. 3) and not found in ac-2, two, Trp 88 and Pro 313, are located in the loop portion of C_λ. Gaps have been inserted in the ac-2 sequence around these positions (Fig. 2). Therefore, these loops, if present in ac-2, may be shorter than the corresponding segments in Ig. In the light of the above, the ac-2 fragment of HLA-B7 has the potential of folding in a manner similar to that in Ig domains. Such a suggestion is in agreement with the high percentage of β-pleated sheet in the HLA-B7 antigen as seen by circular dichroism[37].

The matrix of differences[44] derived from the alignments in Fig. 2 is shown in Table 1. From these data it may be seen that ac-2 displays the same pattern of differences in its relation to all Ig sequences as does any other sequence in Fig. 2. Thus, ac-2 is not related to any particular Ig domain. The ac-2 fragment and β_2m are slightly more different from Ig domains than the latter are among themselves. Also, ac-2 and β_2m are marginally more similar to each other than they are to Ig domains. As it is not clear whether such differences are significant, it may only be concluded that the Ig–HLA and β_2m–ac-2 divergences occurred around the time of the C_H–C_L divergence and C_H domain duplication[32].

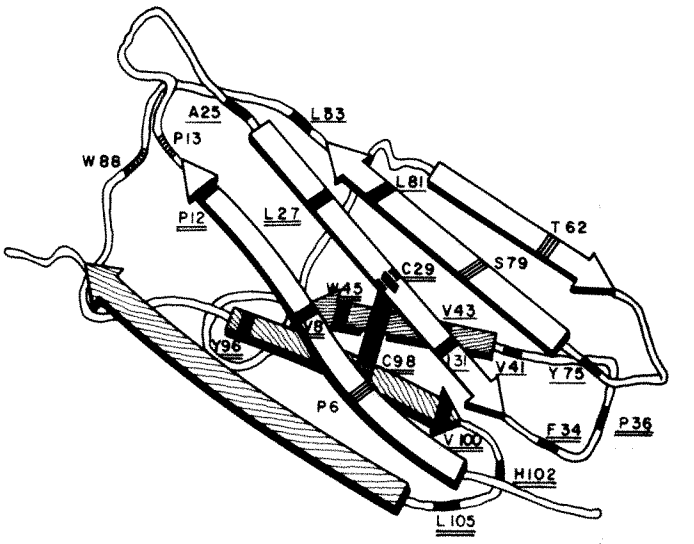


Fig. 3 The folding scheme of a constant Ig domain, as obtained by X-ray crystallographic studies of the Mcg λ human light chain dimer[36]. Arrows denote the location and direction of strands in the β-pleated sheet structure (open, the four-stranded sheet; hatched, the three-stranded sheet). The dark bar between positions 29 and 98 represents the intradomain disulphide bond. Numbering is according to Fig. 2. Single letter coded[44] residues are those appearing in the Mcg light chain sequence, except L105 which appears in most Ig constant domains and ac-2 but is deleted in Mcg. Solid bars denote residues that are invariable in all or most Ig constant domains[32] and appear in the second disulphide region of HLA-B7 (doubly underlined) or restricted to a group of hydrophobic residues in all of the above (underlined). Residues which are highly conserved in Ig constant domains[32], but different in the second disulphide region of HLA-B7, are indicated by the hatched bars. Residues identified by the single letter code[44] are from the Mcg sequence.

To establish the statistical significance of the homology between ac-2 and Ig constant domains, the Z factors (as defined in ref. 28) for some pairs of sequences in Fig. 2 were calculated (Table 2). Such a procedure was used to classify proteins into superfamilies representing a minimal level of relationship between sequences[31]. Values $Z > 6$ suffice to indicate that any two sequences belong to the same superfamily, while $Z > 3$ is sufficient when some functional relationship is known to exist between two proteins[31]. The Z values obtained for the ac-2 fragment with β_2m and seven Ig constant domains are greater than 6 (Table 2). Thus, ac-2 is a new member of the previously established superfamily of Ig constant domains and β_2m[28]. The Z values of ac-2 with a variable Ig domain are as low as those found in comparisons of Ig constant with variable domains (Table 2 and ref. 28).

Fig. 2 The alignment of the ac-2 fragment of HLA-B7 with the human-derived sequences of the superfamily of Ig constant domains and related proteins. Ig sequences from other species were not included since interspecies differences are smaller than those between different classes or homology regions[32]. A computer program AL04 (equivalent to ALIGN, ref. 30) was used, equipped with a core memory saving modification that enabled us to run it on a Data-General Nova 1200 minicomputer. AL04 is based on the algorithm of Needleman and Wunsch[45] which affords the systematic introduction of gaps in both sequences so as to maximise the homology score. As a criterion for the 'goodness of fit' of each matched pair of residues, a scoring matrix[27] was used, whose elements are related to the tendency of those residues to replace each other in many homologous proteins, as well as to their respective abundances and mutabilities. The actual matrix was an updated version for an evolutionary distance of 256 accepted point mutations per 100 residues[46]. In the alignments presented, gap formation was controlled by a penalty of 2 for each break and a bias factor of 2 added to all matrix elements (compare ref. 30). As in all cases gaps appeared only in ac-2, it was possible to reproduce here both the sequences and the numbering system of alignment 27 of ref. 32. The ac-2 sequence was added based on representative alignments following the majority where differences occurred. Boldface letters signify residues at which a particular sequence is identical to ac-2 (ac-2 residues are all boldface irrespective of identities). The number of identities scored by each sequence is given in the extreme right column. Names of sequences designate the Ig heavy chain class and the homology region (for the γ chain, those are 1, 3 and 4). Conserved residues in the Ig sequences, including β_2m, are indicated in *a* by (−), occur in 6–7/12; (=), occur in 8–9/12; (≡), occur in 10–11/12 and (≣), occur in 12/12 sequences; *b*, (▲), indicates positions of hydrophobic residues (Phe, Ala, Tyr, Pro, Met, Val, Leu, Ile or Cys) whose side chains are found to fill the internal space in a folded Ig domain[33]; (*) indicates that an identical (*a*) or homologous (*b*) residue occurs in ac-2. Residues are indicated using the single letter code[44].

Table 2 *Z* factors

	ac-2	β_2m	C_κ	$C_\gamma 4$
β_2m	8.4			
C_κ	8.2	4.1		
$C_\gamma 4$	10.0	6.7	13.6	
C_λ	11.1	—	16.0	—
$C_\gamma 1$	6.9	—	—	—
$C_\mu 3$	7.2	—	—	—
$C_\mu 4$	9.2	—	—	—
$C_\varepsilon 4$	6.4	—	—	—
V_κ	0.6	1.1	1.9	1.2

The statistical alignment scores for ac-2 and several Ig domains. The value of the statistical score, Z, is calculated according to ref. 28. In short, alignment scores were calculated as described in Table 1 legend for the pairs of sequences in question. Then, using the same alignment procedure and gap-controlling parameters, alignment scores were calculated also for one of the sequences with 50 randomisations of the other. $Z = (S - \mu)/\sigma$ where S is the score for the actual protein and μ and σ are the mean and standard deviation for the distribution of scores for randomised sequences. Z is a measure of how likely is a value S to arise by chance; for example, Z equal to 3 and 6 correspond to probabilities of 10^{-3} and 10^{-9} respectively, for this to occur. For comparison some Z values are given for pairs of Ig domains. (—), not determined. V_κ is human AG variable Ig sequence reproduced from ref. 32.

Discussion

The ac-2 fragment of the heavy chain of HLA-B7 is approximately the same size as an immunoglobulin constant domain and, like the latter, contains a disulphide loop of 55 residues. The data presented here establish unequivocally that the heavy chain of HLA-B7 contains an Ig-like region. The features which relate the ac-2 sequence to that of Ig constant domains are summarised as follows: (1) The number of amino acid residues between the two cysteines is very similar to that found in Ig constant domains. (2) They are identical at the three invariant residues—the two cysteines and a tryptophan. (3) They have a high degree of homology at conserved hydrophobic residues. In Ig, some of these participate in the formation of clusters of hydrophobic side chains which are thought to be important in chain folding. (4) More homologous residues are found near the two cysteines of ac-2, as is the case when two different Ig constant domains are compared. And (5), short stretches of alternating hydrophobic residues occur and correspond to segments of β-pleated sheets in Ig. The latter points suggest that there may also be three-dimensional similarities between ac-2 and Ig constant domains. (6) Statistical analysis of the homologies between the ac-2 fragment of the HLA-B7 and Ig constant domains clearly show them to be members of the same superfamily, as defined previously[28]. Such an assessment is the first quantitative indication that the HLA-B7 heavy chain is related to Ig.

One interesting conclusion which can be drawn from the sequence data is that there is an evolutionary relationship between the second disulphide loop region of HLA-B7 heavy chain and Ig constant domains. The occurrence of an Ig-like region in an MHC product suggests that the humoral and cellular arms of the immune system share a common ancestral development. Since cellular immunity phylogenetically predates humoral immunity[38], it would be extremely interesting to determine if primitive cellular responses involve HLA-A and -B-like proteins and eventually explore the structural nature of such proteins. Such studies should lead to a better understanding of the ancestral relationships between the two arms of the immune response. The sequence homology data presented here suggest that the HLA-Ig divergence as well as that of ac-2 and β_2m occurred around the time of Ig C_L and C_H divergence.

The presence of an Ig-like region in the heavy chain of HLA-B7 may have implications concerning the organisation of the genetic material encoding this polypeptide. In the mouse, a two gene–one polypeptide arrangement has been suggested for the H-2K and H-2D antigens. Both limited sequence data[39] and the sharing of a public specificity by each of the private specificities of a given haplotype[40] have been taken to indicate a two gene–one polypeptide arrangement for histocompatibility antigens. Considering the current state of knowledge of the organisation of Ig genes[41,42], the Ig-like region also adds credence to the possiblity that a similar two gene–one polypeptide arrangement may exist for HLA-A and HLA-B heavy chains. The final elucidation of such questions must await additional amino acid sequence data of several specificities from multiple species and studies at the DNA level.

Using a tentative sequence of ac-2[43], A. Munro and A. Feinstein, independently (personal communication) noted that this region of HLA-B7 has structural similarities to an Ig constant domain.

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letters

A compact group of four QSOs with two appearing physically associated

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The objective prism plates from the UK Schmidt telescope taken under good seeing conditions can reach to about magnitude +20 and provide ideal material for studies of the distribution over the sky. We have carried out a detailed search of one of these plates centred on RA 11 h 40 min, dec. 11°00′ N in a preliminary investigation of QSO clustering on scales up to several arc min. Most of the 200 or so QSO candidates noted during this search are emission line objects and definite QSOs but several show no obvious emission features and were selected solely on the basis of their noticeably blue continuum spectra. Subsequent high-resolution spectroscopic observations show that while some of these blue continuum objects are low redshift QSOs a significant fraction are white dwarfs and blue compact galaxies. Allowing for these misidentifications we estimate that a total of 200 QSOs (~six QSOs per square degree) is a conservative upper estimate for the number of genuine QSOs to be found on this plate using our particular selection criteria. With a surface density of about six QSOs per square degree, pairings of QSOs at separations of several arc min are relatively common on a single plate and an individual pair is significant at the 1% level only at a separation of $\lesssim$10 arc. To be considered significant in its own right, a single association at separations of the order of 1 arc min requires a grouping of at least three objects. We report here the most striking association we noted satisfying these conditions, a compact group of four QSOs within a diameter of about 4 arc min. A physical association between two of the QSOs is suggested.

The field of this group of QSOs (denoted a, b, c and d) is shown in Fig. 1. The fifth object (e) is the radio QSO 1146+111 which has a redshift $z = 0.86$ (ref. 1); it shows no obvious emission features and from the objective prism data alone would have been considered only as a possible QSO candidate. There are no other probable QSO candidates in the area shown. Positions accurate to 1 arc min are given for all five QSOs in Table 1 together with O-magnitudes measured from the Palomar Sky Survey prints[2]. Adopting a value of 200 for the total number of QSOs in the 36 square degree plate and assuming them to be randomly distributed, the estimated probability of finding, on this one plate, four or more QSOs within a 12 square arc min area is $\lesssim 6 \times 10^{-5}$. This represents only about a 10% probability of finding such a grouping in a search of the whole sky and strongly suggests that the group is by no means a chance association. An objection to this estimate is that it is strongly influenced by the choice of a 12 square arc min area which has been chosen to fit the particular association. However, when the group was first discovered, the main reason for our believing that the association could be physically significant was the remarkable similarity of the objective prism spectra of a, b and c, all three of which show a single emission feature at roughly the same position in the spectrum, thus suggesting that they might have similar redshifts. Object d, however, which shows two clear emission lines which were identified as Ly-α and C IV at a redshift $z \approx 2$, had clearly a different redshift to other members of the group.

Spectroscopic observations on the five objects in Table 1 were carried out in January 1979 with the 200-inch telescope on Mount Palomar. The results of these observations are summarised in Table 2. Some preliminary observations at the Anglo-Australian Telescope in February on b, c and d gave results which agree with the Palomar observations but observations scheduled in April to obtain more detailed spectra were unfortunately clouded-out. The spectroscopic observations confirm the general inferences drawn from the objective prism data (although object a has clearly varied in brightness since the epoch of the Sky Survey), and strongly support the idea that objects a and c at least do not represent a chance association. Although the signal-to-noise ratios of our data are too low for an accurate comparison of their spectrum, the separation of 2.5 arc min is far too large to allow them to be interpreted as gravitational lens images, the favoured interpretation of the pair of QSOs 0957+561 A, B, which also have identical redshifts but are separated by only 6 arc s (ref. 3). The best redshift estimates

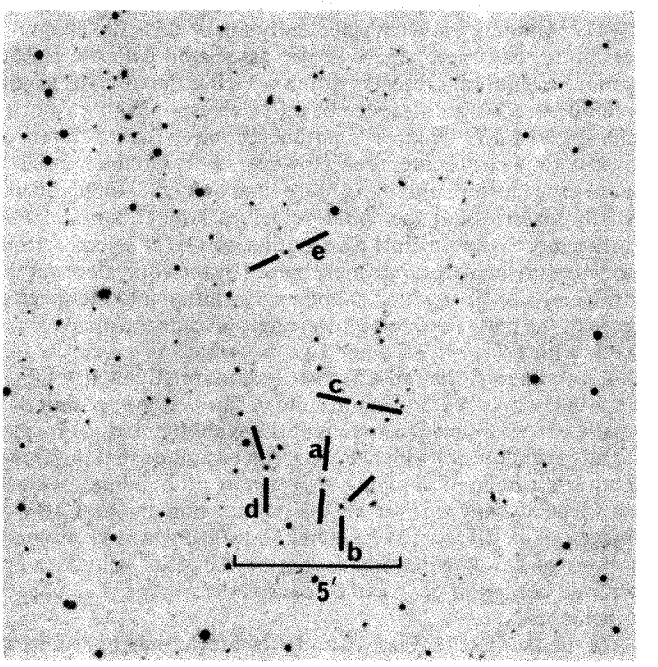


Fig. 1 An enlargement from the National Geographic Society–Palomar Sky Survey O-print of the region around the group of QSOs (a, b, c and d). Object e is the radio QSO1146+111. The 5 arc min scale shown corresponds to the angle subtended by 1 Mpc at a redshift $z = 1$. ($H_0 = 100$ km s^{-1} Mpc^{-1}, $q_0 = 1$). Reproduced courtesy Hale Observatories.

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Table 1 Positions and magnitudes (epoch 1950) from the Palomar Sky Survey Prints

Object	Right ascension			Declination			O-magnitude*
	h	min	s	h	min	s	
a	11	46	09.84	11	04	38.0	18.5
b	11	46	07.58	11	03	49.4	17.9
c	11	46	04.92	11	06	57.1	18.6
d	11	46	16.96	11	05	05.8	17.6
e	11	46	13.42	11	11	39.9	17.9

* Magnitudes are accurate to ±0.3 mag.

Table 2 Spectroscopic data

Object	Magnitude* (V_{5000})	Redshift z	Emission lines observed
a	19.5	1.01 (1.010±0.001)†	C III], [C II], Mg II
b	18.0	1.10	C III], [C II], [Ne IV], Mg II
c	18.9	1.01 (1.011±0.001)†	C III], [C II], [Ne IV], Mg II
d	18.1	2.12	Ly-α, O I, [O IV], C IV, C III]
e	18.5	0.86	[C II], Mg II, He II

* Because of poor photometric conditions the magnitudes are accurate only to a few tenths of a magnitude.

† The figures in braces are the best redshift estimates for objects a and c.

for a and c give a maximum projected velocity difference of 450 km s^{-1}, while the angular separation of ~2.5 arc min corresponds to a projected physical separation of 500 kpc (H_0 = 100 km s^{-1} Mpc^{-1}, $q_0 = 1$). These are typical values for cluster galaxies and the natural inference is that a and c are both members of the same galaxy cluster. Their discovery therefore indicates that the QSO phenomenon is not confined to one highly pecular galaxy in a cluster.

The status of object b is not clear, for the probability of finding an additional two QSOs within a 5 arc min circle (~1 Mpc at $z = 1$) containing a and c is sufficiently small ($\lesssim 5 \times 10^{-4}$) that the possibility that one of these is also associated with the cluster cannot be ignored. Object b with $z = 1.10$ is the obvious candidate but a redshift difference of 0.09 corresponds to a velocity difference of 13,000 km s^{-1}, an unacceptably high differential velocity for a cluster member. However, as T. Gold has pointed out to us, a cluster containing more than one QSO might be unusually massive and the virial velocities larger than average. Furthermore, the redshift distribution of QSOs selected from objective prism plates is strongly biased by the equivalent widths of the emission lines which happen to be shifted into the spectral range of the plates and this bias strongly favours the discovery of QSOs with $1.7 \lesssim z \lesssim 3.2$ when Lyα is visible. As a result of this bias and the intrinsic redshift distribution, relatively few QSOs are found with $z \lesssim 1.7$. That in spite of this bias the redshift of b is within 10% of that of a and c is perhaps another indication that it is not a random coincidence. As it can be argued that this is just another example of applying '*a posteriori* probabilities' we will not speculate any further on this matter nor on the status of object e. The discovery of further statistically significant associations of QSOs with similar large differential velocities are required before object b can be considered other than a chance coincidence.

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IUE UV spectra of the clumpy irregular galaxy MKN297

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Clumpy irregular galaxies are made up of 5–10 bright clumps loosely scattered in a common envelope, have excess near-UV radiation and are giant compared with classic irregular galaxies[1,2]. Clumps are apparently ~100 times more massive than giant H II regions such as 30 Doradus or NGC604 (ref. 3). To get an insight into their properties we have obtained UV spectra using the IUE satellite of one of the clumpy irregular galaxies, MKN297. The UV continuum spectrum thus obtained follows a power law and is steeper (fainter at short wavelengths) than that of a classic irregular or late galaxy. The 155 nm intrinsic luminosity is 400 times larger than that of 30 Doradus. The observed absorption lines can be attributed to early O or B stars, and no emission lines are detectable. We report here that the spectra indicate that each of the clumps contain ~10^4 early stars and are supergiant H II regions in which exceptional bursts of star formation take place.

We have obtained two low dispersion spectra (0.6 nm resolution), one at short (λλ 115–195 nm) and one at long (λλ 190–320 nm) wavelengths, using exposure times of 290 and 310 min. The 10×20 arc s slit was used at PA 27°. The central clump, about half of each of the two next strongest clumps and a few other fainter clumps fell within the field of view of the slit. Without a photometric map of MKN297 it is difficult to evaluate the proportion of light which enters the slit; from Arp's photograph[4] we assume that 50% light and five clumps are present.

The main features of MKN297 (NGC6052) which have already been investigated[3,5–12] are given in Table 1.

Figure 1 shows the UV spectrum smoothed with a 5 nm band and Fig. 2 shows two unsmoothed sections. The continuum has the shape of a power law; when corrected for absorption in our Galaxy with Nandy *et al.*[13] reddening curve, the spectral index is −1.69. It is steeper than those observed for late galaxies or classical irregular galaxies, such as the Large Magellanic Cloud[14] and NGC4449 (ref. 15).

The colours, neutral hydrogen content, Hβ flux and radio continuum flux densities allows a few magnitudes to be estimated for the internal absorption in MKN297; however, these values are not enough to flatten the spectrum.

A steep UV spectrum can be accounted for by an *ad hoc* star population. The absorption lines in our spectrum indicate that

Table 1 Physical properties of MKN297

				Refs
Observed velocity	V_x	4,700	km s^{-1}	12
Galactic extinction in B system	A_B	0.31	mag	18
Visual magnitude	V	13.18	mag	12
Colours	$B-V$	0.44		12
	$U-B$	−0.37		12
	$V-R$	0.56		12
H I flux	F_H	2.5	$10^6 \odot$ Mpc^{-2}	3
Apparent diameter	a_0	2.0	arc min	3
Distance	D	64	Mpc	
Absolute Hβ luminosity		24.0	10^{40} erg s^{-1}	11
Absolute [O III] luminosity		71.6	10^{40} erg s^{-1}	11
Flux density at 1.4 GHz		166	mJy	10
Flux density at 10.5 GHz		18	mJy	9

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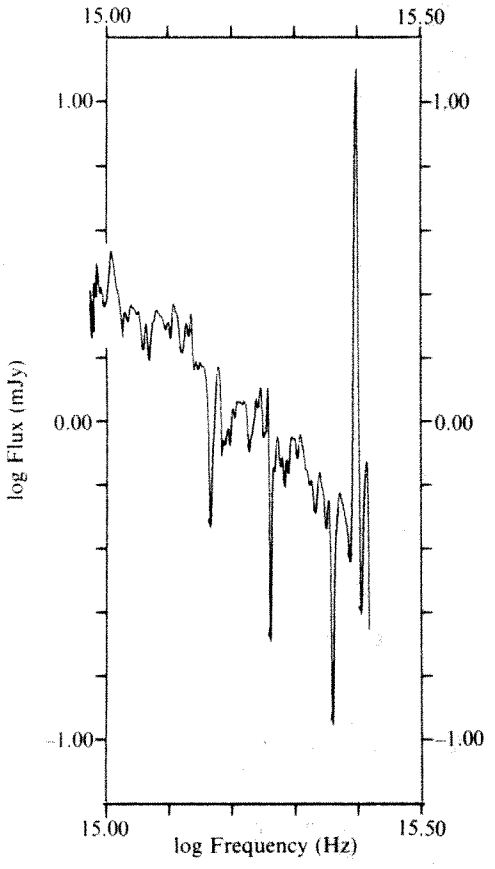


Fig. 1 Smoothed UV spectrum of MKN297. The high frequency spike is due to geocoronal Lyα and the three strong absorptions are reseau marks.

they are produced by stars earlier than AO and, though more like class V stars, they do not exclude class I. Thus, using UV spectra from the Jamar *et al.*[16] Catalogue, corrected for galactic absorption[13], we see in Fig. 3 that fits to the UV spectrum of MKN297 can be obtained by: (i) 3.0×10^4 07 V stars at the high frequency end; (2) 1.3×10^9 A5 V stars combined to the above 07 V stars for wavelengths up to 200 nm; (3) 1.5×10^5 B5 to B8 I stars for most of the UV range.

An attempt has been made to fit the spectrum with a population of 07 and AO V stars plus an internal reddening. The main conclusion is that the stellar flux cannot be reddened using the 'normal' curve of Nandy *et al.*[13] because, even at low colour excesses, it produces a detectable 2,200 Å absorption that is not present in our spectrum. This can be explained if the 2,200 Å

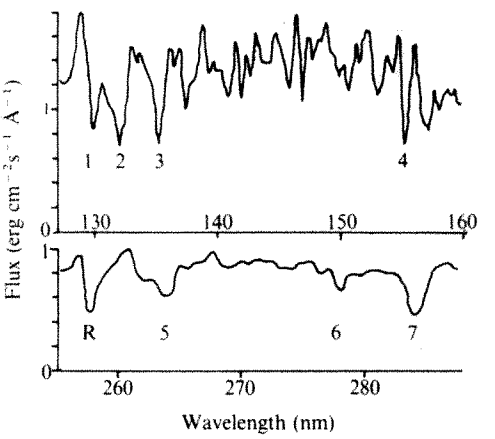


Fig. 2 Two sections of the 'net' spectrum in the short and the long wavelengths. The numbers 1–7 are those given in Table 2 for identification purpose. *R* is a reseau mark.

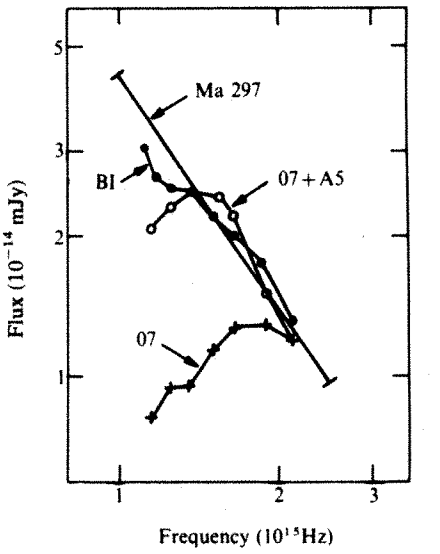


Fig. 3 The UV spectrum of MKN297, corrected for absorption in our Galaxy (straight line) compared with the different star populations given in the text.

feature were mainly due to dust scattering and if we took into account that we are observing the whole region where the scattering takes place. Allowing for this, a reasonable fit has been obtained with a mixture of 4×10^5 07 stars, 2.5×10^9 AO V stars and an internal reddening of $E^i(B-V)\sim0.5$.

Therefore, a clump apparently contains $\sim10^4$ early stars.

An interesting result is obtained by comparing the UV emission of MKN297 with that of the giant H II region 30 Doradus. Using the 155 nm map of 30 Dor obtained by Korneef[17], the de Vaucouleurs *et al.*[18] galactic absorption and the Nandy *et al.*[13] curve one gets a flux density of 30 Dor corrected for galactic absorption which, when transferred from its 50 kpc distance to that of MKN297, is 400 times less than the one we observed for MKN297. A clump is thus ~100 times more powerful than a giant H II region.

Absorption lines together with an indication of their quality are listed in Table 2. Two lines may be attributed to interstellar absorption in our galaxy: O I λλ130.1 and 135.2 and C IV λ155.0. nm. The others correspond to the redshift of MKN297.

Spectral features typical of stars from late O to AO are visible: Si IV λ140.0, C II λ133.5, C IV λ155.0, Fe II (8) λ162.0, Fe II (3) λ234.0 nm.

Table 2 Identification of absorption lines in the UV spectrum of MKN297

Measured λ(Å)	Corrected λ(Å)	Identification (main contributors)	λ_0(Å)	No. in Fig. 2	Comments
1,238	1,219	Lyα	1,216		
1,256	1,236	N V	1,241		
1,301	—	O I	1,302	1	Galactic
1,321	1,300	Si III	1,299	2	
1,352	1,331	C II	1,335	3	
1,408	1,386	Si IV:	1,398		Doubtful
1,552	—	C IV	1,549	4	Galactic
1,570	1,546	C IV; Si II:	1,549; 1,533		
1,646	1,620	Fe III; Al III; Fe II; He II	1,600÷1,640		Broad feature
1,701	1,675	Al II; Fe II	1,671; 1,675		
1,922	1,892	Fe III	1,886÷1,897		Several multiplets
1,948	1,918	Fe III:			Doubtful
2,026	1,994	Fe III:			Doubtful
2,340	2,304	?			Doubtful
2,373	2,336	Fe II; Cr II	2,340		Several multiplets
2,622	2,582	Fe II	2,578		Broad feature
2,640	2,599	Fe II	2,599	5	Broad feature
2,783	2,740	Fe II	2,739	6	
2,841	2,797	Mg II	2,799	7	
2,887	2,843	Mg I	2,852		

The Fe II and other singly ionised metal feature at λ240.0 which is a strong feature in stars later than AO V is not visible. The Mg II λ280.0 and Mg I λ284.0 nm lines are not particularly strong, suggesting also types not later than A.

In the visible, du Puy and de Veny[5] observee Ca II λ393.3 nm and some Balmer lines, which also correspond to early types.

The identifications are consistent with the presence of hot main sequence stars. The λ234.0 line favours class I–II stars, but has also been observed in class IV stars[19]. Other features corresponding to bright stars, Fe III (51) λ192.0 and the wide dip from λ180 to 215 (ref. 20) are dubious.

The presence of λ155.0 and the absence of λ240.0 are also consistent features for class I stars.

Using the criteria of Jaschek *et al.*[21], the r_1, r_2, r_3 values calculated from flux densities at λλ141.0, 155.0, 158.0, 162.0 nm after correction for absorption in our galaxy yield an integrated spectral type B5 V. A further internal absorption $E^i(B-V)=0.6$ would yield a type B2.

The r_5 ratio of flux densities at λλ240.0 and 274.0 nm of Cucchiaro *et al.*[22] for A stars leads also to a faint luminosity class.

There are no emission lines in our UV spectrum of MKN297, contrary to the visible case[5,6]. With the hypothesis that the gas in the clumps has physical conditions similar to those of H II regions, the expected intensity ratio betweeen, for example, the C III] 190.7–190.9 nm lines and the [O II] 372.7–272.9 ones is found to be too small to give a detectable signal at ~191 nm.

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Accretion powers the brightest stars

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The brightest known stars in external galaxies are the Hubble–Sandage variables. These are normally assumed to be hot, massive, single stars evolving either towards or off the main-sequence. An alternative model is developed here involving binary mass transfer and accretion on to main-sequence stars. In this case the Hubble–Sandage variables are a third, and presumably final, class of binary in which accretion generates the bulk of the radiation observed. The other two classes are binaries containing an accreting white dwarf (cataclysmic variables)[1,2] and an accreting neutron star or black hole (binary X-ray sources)[3,4]. Accretion disk models developed in these two cases can be scaled directly to treat the main-sequence case. One of the most complex objects in our own Galaxy is the η Carina nebula, rivalling in this respect the Orion Nebula and the Crab. I suggest below that η Can itself is an accreting main sequence star whose explosive outburst in the nineteenth century was caused by runaway mass accretion.

The Hubble–Sandage variables form a class of luminous, blue, irregular variable stars. In the original Hubble–Sandage study[5] five variables in M31 and M33 were investigated to determine their group properties for use as distance indicators. The spectroscopic properties of these variables have been extensively studied[6,7], and the optical variability of eight group members has been discussed elsewhere[8].

The main class characteristics are high luminosity, blue colour indices and irregular variability on time scales ~1 yr with amplitude ≲2 mag. The maximum visual magnitudes are between −8 and −11 (visual luminosities $\sim 10^{39}$ erg s^{-1}). Gravities are low[6]. The spectra exhibit strong UV continua, no Balmer discontinuity and emission lines of H and He I, with generally weaker Fe II and [Fe II]. In this respect they closely resemble cataclysmic variables, particularly dwarf novae at quiescence. Dwarf novae show broad H and He I together with a strong UV continuum extending in some cases down to 1,250 Å (refs 9, 10). Weak Fe II in emission is also observed[9]. The Balmer jump is either not observed, or, more rarely, is present weakly in emission[9,11]. The principal difference is the increased breadth of the lines in dwarf novae, which are occasionally double. In contrast to the spectral similarities the luminosities of cataclysmic variables could hardly differ more. Rather than being the brightest stellar objects they are fainter than the Sun with luminosities $\sim 10^{32}$–10^{33} erg s^{-1}.

The general spectral similarity and the extreme disparity in luminosity suggest that similar physical processes occur in each class, but on differing scales. In quiescent novae the spectrum is generated by infall of gas from a companion onto a white dwarf mediated by an accretion disk. The Hubble–Sandage variables would result from similar physical conditions being generated but scaled to larger radii so that a much higher luminosity is generated. The occasional nova-like outbursts in Hubble–Sandage variables supports this identification.

I assume the Hubble–Sandage variables contain an accreting main-sequence star in a mass-transferring binary with a separation $\gtrsim 10^{12}$ cm. This separation is needed to accommodate a Roche lobe-filling companion such that an accretion disk may be formed. With a main-sequence star the major changes affecting accretion result from the much reduced gravitational potential at the stellar surface, and the possibility of central masses exceeding $\sim 2M_\odot$. Recent work[12–14] shows that with high accretion rates the main-sequence star will expand, often dramatically. Such expansion would require higher accretion rates to generate disk conditions similar to those derived below for normal main-sequence stars.

For an accretion rate $\dot{m}$ g s^{-1} the gravitational luminosity, $L = GM\dot{m}/R$. The characteristic source temperature is roughly the black-body temperature, $T = (L/4\pi\sigma R^2)^{1/4}$. For a main-sequence star to be at the same temperature as an accreting white dwarf and hence have the possibility of exhibiting similar general spectral features, the accretion rate must be increased by a factor $\dot{m}_{MS}/\dot{m}_{WD} = (R_{MS}/R_{WD})^3$ $(M_{WD}/M_{MS}) \sim 10^5$–$10^7$ (for masses $1 < M_{MS}/M_\odot < 100$) and the ratio of luminosities is then $L_{MS}/L_{WD} = (R_{MS}/R_{WD})^2 \sim 10^4$–$10^6$. Thus an accreting main-sequence star with disk temperatures similar to those of quiescent cataclysmic variables will have a disk luminosity $\sim 10^{36}$–10^{39} erg s^{-1}.

I now examine this model using steady disk models (the consequences of time dependence are unimportant). Assuming the disk is optically thick, which is valid so long as $T > 10^4$ K, then[4]

$$T(R) = \left(\frac{3GM\dot{m}}{8\pi\sigma R_{MS}^3}\right)^{1/4}\left(\frac{R}{R_{MS}}\right)^{-3/4}\left(1-\left(\frac{R}{R_{MS}}\right)^{-1/2}\right)^{1/4}$$

The maximum disk temperature occurs at a radius 40% larger than R_{MS} and is given by

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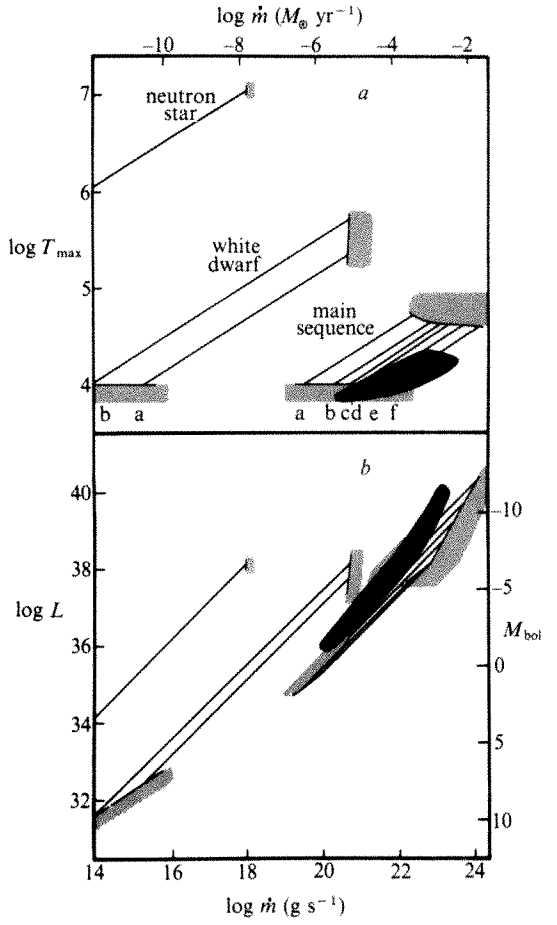


Fig. 1 Variation of maximum disk temperature (*a*) and disk luminosity (*b*) with accretion rate. Accretion tracks are shown for 0.5(a), 1(b), 3(c), 9(d), 30(e) and 121(f) solar mass main-sequence accretors. The region in which the accretion luminosity is less than the nuclear luminosity is indicated by heavy shading. At the upper boundary the disk luminosity is equal to the Eddington limit and the disk disrupts. At the lower boundary disk temperatures are less than $\sim 10^4$ K and the disk becomes optically thin. Tracks for white dwarfs of 0.4(a) and 1(b) $M_\odot$ and a neutron star of 1 $M_\odot$ are also shown.

$$T_{max} = 0.488\left(\frac{3GM\dot{m}}{8\pi\sigma R_{MS}^3}\right)^{1/4}$$

$$= 8.3\times 10^3 \dot{m}_{20}^{1/4}\left(\frac{M}{M_\odot}\right)^{1/4}\left(\frac{R_{MS}}{R_\odot}\right)^{-3/4}$$

where $\dot{m}_{20} = \dot{m}/(10^{20}\,\mathrm{g\,s^{-1}})$. Disk temperatures are hotter than 10^4 K for accretion rates $>10^{20}\,\mathrm{g\,s^{-1}}$.

Figure 1*a* shows the variation of T_{max} with $\dot{m}$ for main-sequence stars with masses in the range 0.5–121$M_\odot$. (Radii are from refs 15–17.) For comparison disk temperatures are also shown for white dwarf and neutron star accretion.

Cut-offs exist at both low and high temperatures. With low accretion rates and temperatures $<10^4$ K the rapid fall in opacity associated with hydrogen recombination leads to the possibility that the disk is optically thin. In these conditions radiation will be emitted in emission lines, with no strong continuum. In the case of massive early-type accretors a second cut-off exists at low temperatures. For $M > 9M_\odot$ the nuclear luminosity can be greater than the accretion luminosity for temperatures $>10^4$ K.

At high temperatures disk solutions break down when the luminosity becomes supercritical[4] and radiation pressure disrupts the disk. This occurs when $L > L_{ed} = 4\pi GMc/K$. This upper boundary is shown in Fig. 1*a* for an electron scattering opacity K, and a hydrogen abundance $X = 0.7$. The associated luminosity changes are shown in Fig. 1*b*.

It is evident that accreting main-sequence stars give a satisfactory model of Hubble–Sandage variables. With accretion rates 10^{20}–$10^{24}\,\mathrm{g\,s^{-1}}$ disk temperatures are the same (1–4 × 10^4 K) as quiescent dwarf novae. Luminosities, on the other hand, are $\sim 10^{38}\,\mathrm{erg\,s^{-1}}$. To achieve the highest luminosities ($>10^{39}\,\mathrm{erg\,s^{-1}}$) massive main-sequence stars ($>9M_\odot$) are required, with accretion rates $\sim 10^{23}\,\mathrm{g\,s^{-1}}$. Massive main-sequence accretors will form the brightest Hubble–Sandage variables.

Are other physical properties, besides temperature, such as optical thickness and density, similar for both main-sequence and white dwarf accretion? The optical depth, τ, at temperatures $\sim 3\times 10^4$ K is given by Sunyaev and Shakura's regime c[4], where

$$\tau = 7.5\times 10^2 \alpha^{-4/5}\dot{m}_{20}^{1/5}\left(1-\left(\frac{3R_g}{R}\right)^{1/2}\right)^{1/5}$$

Here α is the disk viscosity parameter, and R_g the Schwarzschild radius. As $\alpha < 1$, and $R_g \ll R$, $\tau > 7.5\times 10^2 \dot{m}_{20}^{1/5}$. With $\alpha = 1$ main-sequence accretors have $\tau \sim 10^3$ and quiescent cataclysmic variables have $\tau \sim 10^2$. Both are optically thick and will have a similar appearance.

The surface density, Σ, of the disk is given by[4],

$$\Sigma = 9.8\times 10^{-6}\alpha^{-4/5}\dot{m}_{20}^{7/10}\left(\frac{M}{M_\odot}\right)^{1/2}$$

$$\times\left(\frac{3R_g}{R_*}\right)^{3/4}\left(1-\left(\frac{3R_g}{R}\right)^{1/2}\right)^{7/10}\mathrm{g\,cm^{-2}}$$

Thus

$$\Sigma > 2.9\times 10^{-11}\dot{m}_{20}^{7/10}R^{-3/4}\left(\frac{M}{M_\odot}\right)^{1/4}\mathrm{g\,cm^{-2}}$$

For the case $\alpha = 1$ main-sequence stars typically have $\Sigma \sim 10^{3\text{–}4}\,\mathrm{g\,cm^{-2}}$ and white dwarfs $\Sigma \sim 10^2\,\mathrm{g\,cm^{-2}}$. Thus density differences may affect the spectrum and could account for the stronger Fe II lines in some Hubble–Sandage variables.

The broadening and doubling of the lines in cataclysmic variables are due to rotational Doppler shifts of material moving in the disk with Keplerian velocities. The maximum velocity at the stellar surface is $V = (GM/R)^{1/2}$, corresponding to an orbital period $P = 2\pi(R^3/GM)^{1/2}$. For main-sequence stars in the mass range $0.5\,M_\odot < M < 120\,M_\odot$, periods are in the range $1 < P < 14$ h and velocities $400 < V < 1{,}200\,\mathrm{km\,s^{-1}}$. For cataclysmic variables, $10 < P < 60$ s and $2{,}000 < V < 5{,}000\,\mathrm{km\,s^{-1}}$. Hence main-sequence line-broadening is 3–10 times less than that occurring in cataclysmic variables and is compatible with the observed difference in emission linewidth. Note that the maximum broadening will occur in the most massive, and most luminous objects. Doubling of lines would support the disk model.

The Balmer jump is absent due to the spectrum being composed of contributions from different radii in the disk, as in cataclysmic variables. The spectrum should obey the $F_\nu \propto \nu^{1/3}$ law[23] observed in dwarf novae[10]. In the two colour diagram both classes lie in roughly the same region, $U-B \sim -1.0$, $B-V \sim 0.0$, close to the black-body line.

The required accretion rates of 10^{20}–$10^{24}\,\mathrm{g\,s^{-1}}$ (10^{-6}–$10^{-2}\,M_\odot\,\mathrm{yr^{-1}}$) can be achieved (in principle) by Roche lobe-filling companions, particularly if these are giants. Variability is due to fluctuations in the mass transfer rate, possibly due to instabilities driven by ionisation zone destabilisation within the companion's envelope.

At accretion rates above the critical luminosity, auto-regulation of the accretion flow sets in and the disk is disrupted in the inner region[4]. This leads to a fraction of the accreting gas being ejected in the form of an outflowing wind. The low gravitational potential at the surface of a main-sequence star means that this wind is likely to be optically thick. It will have the same general properties as the optically thick winds observed in explosions of classical novae[18]—increases in the mass ejection rate will generate expansion of the photospheric radius, a decrease in both effective temperature and the bolometric correction but an

increase in the visual luminosity, with little change in the bolometric luminosity. Outflow velocities will be lower than in classical novae: roughly the same order as the escape velocity from the stellar surface.

Consequently there will be two types of spectroscopic and colour change produced by variations in the accretion rate. In the subcritical regime, increased accretion rates produce increasing disk temperatures along with increasing luminosity—both visual and bolometric. In the supercritical regime the disk is surrounded by an outflowing optically thick wind and the bolometric luminosity is held close to the Eddington value, L_{ed}. Increases in the wind mass-loss rate due to further increased accretion now produce decreasing temperatures along with increasing visual luminosity. The emission line spectrum of the disk will be replaced by an absorption line spectrum formed in the outflowing envelope—similar to the F or A type spectrum observed in classical novae at outburst maximum.

Is there any evidence for this supercritical state? The photographic magnitude of M33 Var A fell by 3.5 mag (ref. 5) in the visual in 1950–52, rose by 1.0 mag in 1952, before finally falling off sharply. Hubble and Sandage note that the changes in colour index indicate that the bolometric luminosity changed by only 1.4 mag. The visual luminosity fell by ~60 while the bolometric luminosity fell by ~4. This agrees with the expected changes due to a variable optically thick wind at Eddington luminosities.

η Carina is a close galactic relative to the Hubble–Sandage variables. It has broad spectral lines, with relatively strong Fe II and [Fe II][6]. Davidson[19] deduced a temperature of 3×10^4 K for the underlying star. Between 1836 and 1856 η Carina underwent a major outburst, followed by a subsidiary outburst in 1889. The early F absorption spectrum of 1893 was slowly replaced by the characteristic emission spectrum. The absolute magnitude at outburst was ~-14 (ref. 20), several magnitudes brighter than normal classical novae. At present, a cool surrounding dust cloud[21] heated by the underlying stellar source produces the bulk of the emission. The bolometric luminosity is still ~-14, but most of the emission is re-radiated IR from the dust shell. This high and constant luminosity, the spectral changes, the broader than average emission lines, and the nova-like explosion indicate that η Carina could contain a massive, main-sequence, accreting component in a binary which underwent a burst of supercritical accretion. Polarisation observations[20] of the nebula suggest that the dust is concentrated in an annulus of size 10^4 AU, and this has been interpreted as evidence of binary structure. This is the first observational indication of possible binary structure in this type of object. A search for binary eclipses in Hubble–Sandage variables with periods in the range weeks to years would test the binary model.

The bright galactic source MWC349 is a similar object[7]. Thompson *et al.*[22] interpret optical and IR observations as a proto-planetary disk seen face-on. In terms of the structure of the luminous source their model closely resembles that described here. The main difference is their interpretation as a preplanetary disk rather than an interacting binary.

If the brightest known optical stars are powered by infall of material on to a main-sequence star, the importance of accretion as an astrophysical energy source would be established beyond its present applications to X-ray binaries, novae and active galactic nuclei and quasars[23]. Such binaries should exist, and they would be classified as Hubble–Sandage variables when observed in external galaxies.

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Streaming of interstellar grains in the Solar System

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Deep-space probes offer excellent opportunities to study interstellar grains streaming into the Solar System. Direct detection of such particles would considerably add to our knowledge in many areas of cosmic dust research and could also provide information on the characteristics of both the interstellar medium and the interacting solar wind. Here we present the results of a theoretical study of the interaction between interstellar grains and the solar wind, including the effects of solar cycle variations in the interplanetary magnetic field. The latter is shown to influence significantly regions of concentration and depletion of interstellar grains within the Solar System.

Observations of the neutral gas in the solar vicinity suggest that the Solar System is moving relative to the local interstellar matter in a direction $\simeq7°$ north of the solar equatorial plane (ecliptic longitude and latitude: $\lambda\simeq250°$, $\beta\simeq7°$)[1], and at a speed of $\simeq20$ km s^{-1} (ref. 2). Bertaux and Blamont[3] computed number densities of interstellar grains in the solar cavity taking into account only gravitation and radiation pressure. Of the types of grains used in their model and based on their calculation of β (radiation pressure to gravitational attraction) only 0.02-μm radius silicate grains were able to penetrate deeply inside the Solar System. They estimated the number density of these dust grains to be strongly peaked on a line extending from the Sun backwards in the downstream direction. This number density was found to be higher by a factor of ~100 than direct observations of dust grains by Alvarez[4]. In an attempt to clarify this difference, Levy and Jokipii[5] made an order of magnitude estimate of the Lorentz force exerted by the solar wind magnetic field on a charged grain. They showed that if the 0.02-μm radius silicate grains of Bertaux and Blamont carry a net charge of 5 V, then the Lorentz force strongly dominates, thereby destroying the gravitational focusing and suggesting that these grains are largely excluded from the Solar System.

Studies of interstellar grains suggest two populations of particles[6]: (1) elongated core–mantle particles of characteristic radius $\simeq0.12$ μm, with a $\simeq0.07$ μm radius core of silicate type material, and a rather complex mantle of molecular mixtures of mainly oxygen, carbon and nitrogen; and (2) $\simeq0.01$ μm thick silicate and/or graphite type grains. Bertaux and Blamont did not consider any of these populations. In the following calculations (see also, ref. 13) we show that although population (1) particles have $\beta>1$, they still penetrate deep inside the Solar System.

The magnitude of the radiation pressure is given by $P_{pr}=(S/c)Q_{pr}$, where S is the flux, c is the speed of light, and Q_{pr} is the radiation pressure efficiency: $Q_{pr}=Q_{abs}+Q_{sca}\ (1-\langle\cos\theta\rangle)$, where Q_{abs} and Q_{sca} are the efficiency factors for absorption and scattering, respectively, and $\langle\cos\theta\rangle$ is the asymmetry factor over

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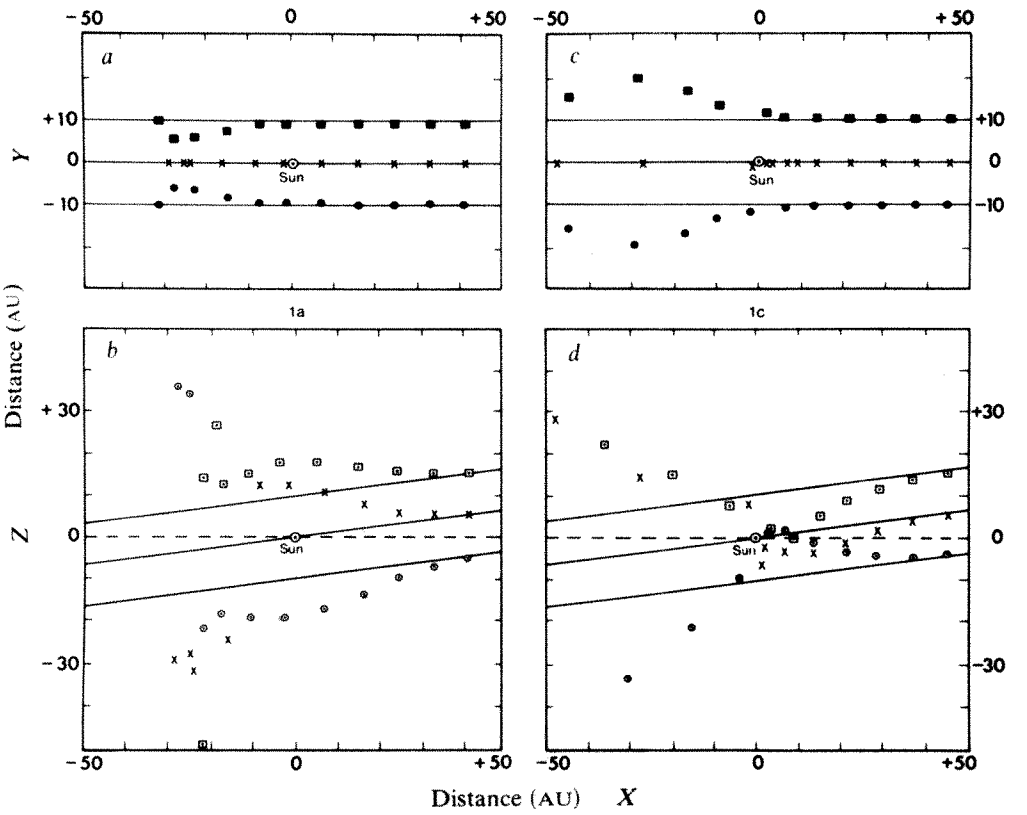


Fig. 1 Instantaneous positions of the 0.12 μm core–mantle particles initially moving in the direction $\lambda = 70°$, $\beta = 7°$ along the solid lines. The particles are entering the Solar System at $X = +50$ AU with 2 yr interval. Three streams (■, ×, ●) introduced at $Z = +6$ AU are projected onto the solar equatorial (X–Y) plane at the beginning (a) and in the middle (c) of a solar magnetic cycle. In b and d the three streams (⊡, ×, ⊙) introduced at $y = 0$ are similarly projected onto a plane (X–Z) perpendicular to the solar equatorial plane (dotted line).

the scattering angle θ. Scattering dominates over absorption for these dielectric particles in the scattering-efficient resonance region, and Q_{pr} was estimated using Mie theory for homogeneous spheres of equal volume and averaged core–mantle index of refraction. For a spherical silicate core coated by a concentric smooth spherical ice mantle representing particles of population (1) the computed Q_{pr} value using this approximation was found to be 0.4 ($\beta = 1.3$). As the grains that penetrate (population (1)) spend 30 yr or less in the Solar System, all radiation forces other than radiation pressure are negligible. Both solar-induced photoelectron emission and the accretion of solar wind ions will tend to charge these grains positively, whereas electron accretion produces negative charge. The equilibrium charge state is given by the relation

$$\sigma_e(V_0)f_e = \sigma_i(V_0)f_i + y\sigma_{ph}f_{ph} \quad (1)$$

where the σs are the cross-sections of the grains for UV photons, ions and electrons, the fs are the corresponding fluxes, y is the photoelectron yield, and V_0 is the electrical potential of the grains. σ and y have been estimated for numerous silicates, and these lead to charges ranging from 5 to 12 V (refs 7, 8). The photoemission properties are not usually decreased by surface impurities or adsorbed layers[7], or by amorphous structure[8]. However, for ice the work function is near the Lyman edge, and therefore photoemission is less efficient. We will adopt here +7 V for bare particles, and +1.2 V for the core–mantle grains. As the charged grains interact with the solar wind described by Svalgaard *et al.*[9], they will be subject to a Lorentz force given by

$$F_L = \frac{q}{c}[(V_g \times B) - (V_{sw} \times B)] \quad (2)$$

where q is the grain's charge, V_g and V_{sw} are the speeds of the grain and of the solar wind, respectively, c is the speed of light and B is the magnetic field strength.

The magnetic field is torn in an archimedian spiral by the solar rotation. Thus

$$B_r = \frac{B_0}{r^2} \quad \text{and} \quad B_\Phi = \frac{-B_0\omega b^2(r-b)\sin\theta}{V_{sw}r^2} \quad (3)$$

where B_0 is the magnetic field strength at some reference distance ($b = 5$–10 solar radii), ω is the solar angular velocity, r is the heliocentric distance, Φ is the azimuth angle, and θ is the heliocentric colatitude angle. The second (electrical) part of equation (2) which acts along θ will dominate as the grains enter the Solar System at low and medium heliocentric latitudes. This force, acting on a grain of given charge, is independent of the speed of a radially streaming solar wind as can be seen by substituting equation (3) in equation (2); high velocity plasma streams will not alter our results considerably.

The positive and negative magnetic fields are separated by a sinusoidal warped neutral sheet[10,11], the amplitude of which we assume to decay exponentially with increasing heliocentric distance. The speed of the solar wind as a function of θ was assumed to be of the form

$$V_{sw} = a + b\,|\cos\theta| \quad (4)$$

where a is the mean solar wind velocity at the equator (400 km s^{-1}) and b is 300 km s^{-1}.

The grains also encounter other less important forces such as Coulomb drag due to the nearby passing solar wind electrons, which is given by

$$F_C = \frac{\pi s^2 N_e e^2 V_0^2}{m_e W^2} \ln\left(\frac{W^2 m_e P}{e V_0 s}\right) \quad (5)$$

where s and V_0 are the radius and charge of the grain, respectively, N_e, e, and m_e are the electron number density, charge and mass, respectively, and W is the velocity of the grains relative to the electrons. P is the impact parameter, which is assumed to be $\approx N_e^{1/3}$, following a poissonian distribution for the nearest neighbour distance. Another force is ion pressure resulting from impinging solar wind ions on the grains which is given by

$$F_i = \pi s^2 \bar{M}_i V F \quad (6)$$

where F is the flux of the solar wind ions, V is their velocity and $\bar{M}_i$ is the mean ion mass $\approx 2 \times 10^{-24}$ g.

Trajectories of interstellar grains streaming into the Solar System were computed as follows: the grains are introduced at the boundary of the solar wind cavity ($X = 50$ AU) at the beginning of the 22-yr solar magnetic cycle (defined as the cycle beginning when the solar dipole field acquires a south component). The equations of motion are integrated at small intervals of time (24 h), and the position, speed and acceleration of the particles are determined.

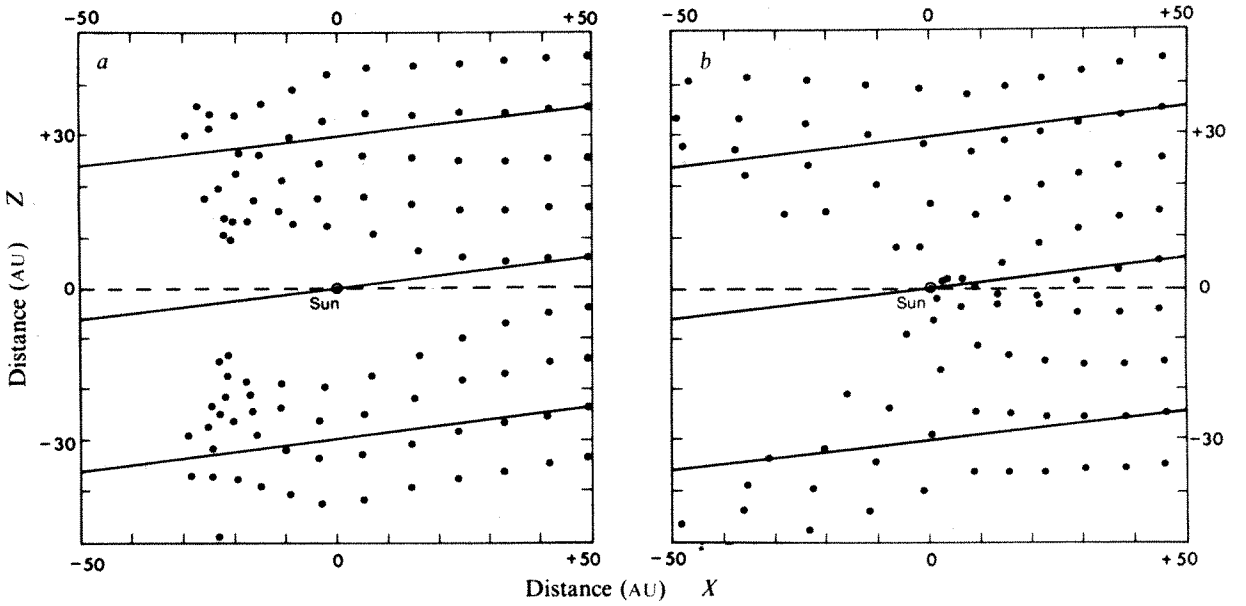


Fig. 2 Instantaneous positions of combined high latitude streams and the streams shown in Fig. 1*b* and *d*, at time zero (*a*) and time 11 yr (*b*) of a solar magnetic cycle.

The results of these computations are shown in Figs 1 and 2. Figure 1*a* and *c* shows the projection onto the solar equatorial plane (XY-plane) of the instantaneous positions of population particles arising from three streams which differ only by a separation of 10 AU in the initial value of their Y coordinate. Figure 1*b* and *d* shows the instantaneous positions in the XZ-plane of particles arising from three streams with $Y = 0$. One stream is initially directed towards the Sun and the other two lie parallel to and 10 AU on either side of that direction. The sequence of points belonging to a stream corresponds to positions of particles which differ by 2 yr from the initial time when each particle was introduced at the boundary of the solar wind cavity. Figure 1*a* and *b* shows the instantaneous positions of all the particles at a time corresponding to the beginning of the solar magnetic cycle. Figure 1*c* and *d* repeat this information for a time 11 yr into the solar magnetic cycle. The contrast between *a*, *b* and *c*, *d* clearly demonstrates the decisive influence of the solar magnetic cycle on the distribution of the grains. The streams are diverted towards the X-axis in Fig. 1*a* and away from that axis in Fig. 1*c*. Figure 1*b* shows that when the solar dipole field axis points north, the grains are diverted from the solar equatorial plane, mainly due to the action of the electrical part of the Lorentz force. The grains are concentrated towards this plane when the solar dipole field points south (Fig. 1*d*). Some 3 to 4 AU ahead of the Sun the streams cross the equatorial plane, creating an enhancement in the number density. The grains behind the Sun experienced the oppositely directed magnetic field of the first half of the solar magnetic cycle as they entered the solar wind cavity, thus creating a depletion zone in the wake of the Sun.

Our computations are almost insensitive to whether the reversal of the direction of the interplanetary magnetic field is instantaneous or whether it is as gradual as a sine function. These results are based on a simple dipole field where higher order multipole or asymmetrical magnetic fields are ignored.

In Fig. 2*a* and *b* additional streams, projected in the XZ plane, are introduced higher in latitude than the streams in Fig. 1*b* and *d*. Figure 2*a* shows a concentration of particles developing along a line $X \approx -20$ AU, $Z \approx \pm 10$–20 AU (peaked around $\phi \simeq 180°$). There is also a depletion zone of radius ≈ 20 AU surrounding the Sun. The deviation from the XZ-plane is $\leqslant \pm$ 0.1 AU. The value of Q_{pr} used in the calculation is 0.4, which might be increased by surface roughness; however, this value decreases for elongated particles. The overall effect of increasing the radiation pressure efficiency Q_{pr} of the grains from 0.4 to as much as 1.0 ($\beta = 3.25$) is to move the concentration in Fig. 2*b* ≈ 3 AU along the positive X-axis. On the other hand, a decrease in the value of Q_{pr} has a negligible effect. The smaller bare grains that enter at low and medium heliocentric latitudes are essentially trapped around the solar wind magnetic field lines, and they are swept out near the solar wind cavity.

We have extended the study of the dynamics of interstellar grains to particles having radii of 0.12 and 0.005 μm streaming into the Solar System and included the effects of the solar dipole field's change of direction during the solar magnetic cycle. Additional forces on the particles such as Coulomb drag and solar wind ion pressure are less important than the Lorentz force. Particle trajectory computations show that an enhancement of the order of 50 or more in the number density of interstellar core-mantle particles may occur some 3–4 AU ahead of the Sun and close to the solar equatorial plane during yr 8–17 of the solar magnetic cycle. During the rest of the cycle, concentrations develop some 20 AU away from the Sun, while a depletion zone of ≈ 20 AU in radius surrounds the Sun.

As particle configuration varies strongly with the solar magnetic cycle, its effect must be taken into account in any treatment of this problem. Note that even grains with $\beta > 1$ will penetrate deep inside the Solar System during part of the solar magnetic cycle.

Subsequent computations may predict more precisely the directions and times to search for an interstellar component of the zodiacal cloud. Note that during the Solar Polar Mission (mid and late 1980s), concentrations of interstellar grains are expected (Figs 1*d*, 2*b*) which will offer a unique opportunity to detect the presence of interstellar grains in the Solar System[12].

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Deposition rate and seasonal variations in precipitation of cosmogenic ^{10}Be

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The measurement of ^{10}Be using nuclear accelerators promises to widen the applications of this cosmogenic isotope considerably[1-3]. A parameter of fundamental importance in such applications is the production rate of this nuclide in the atmosphere. This production rate has previously been estimated by two methods: (1) by measuring the concentration of ^{10}Be in marine sediments having known accumulation rates; (2) by using model calculations of cosmic ray interaction rates, combined with estimated cross-sections for production of ^{10}Be. These two methods have led to an estimated global average ^{10}Be production rate of 0.015–0.018 atoms cm^{-2} s^{-1} (refs 4, 5 and F. Guichard, unpublished work). The increased sensitivity available with the accelerator technique now makes it feasible to measure directly ^{10}Be deposition rates at the Earth's surface. We report here the results of such a measurement, which lead to an estimated global average production rate more than double the previous estimates. We also give some initial results on the seasonal variation of ^{10}Be concentration in precipitation, and comment on the possibility that such variations might permit the determination of ancient accumulation rates of polar ice.

The ^{10}Be for our measurements was collected in a child's plastic swimming pool (area 2.5 m^2) exposed on the roof of the Laboratoire René Bernas (~49° N) between 3 April 1978 and 6 April 1979. Initially 10 mg of ^{9}Be and several litres of water were added to the swimming pool. At the end of the exposure period the swimming pool contained ~700 l. A known fraction (10 l) of this volume was recovered and analysed for ^{9}Be by flameless atomic absorption spectrometry. This allowed us to determine the fraction of the initial ^{9}Be (and thus deposited ^{10}Be) which was recovered. This value agreed very well with the fractional volume of water recovered, showing that essentially all the Be had remained in solution. (This somewhat surprising result can probably be explained by the *p*H of the precipitation, which was ~5.5 at the time of recovery). As well as determining the yearly average by a single measurement, the above method of collection includes any contribution from 'dry' deposition.

Additional ^{9}Be (4mg) was added to the 10-l sample which was then passed through a Chelex ion exchange column on which the Be was retained. The Be was then eluted in 1.5M HCl, and purified by a series of solvent extractions[6]. The Be was finally transformed into a sintered BeO disk by a process described elsewhere[1].

The measurements on the cyclotron were carried out as described elsewhere[2]. An important improvement, however, was that samples and standards could now be mounted on a rotating source holder, which permitted us to change samples remotely in a matter of seconds, without modifying any of the accelerator parameters. Details of this system will be given elsewhere.

From the measured $^{10}Be/^{9}Be$ ratio, the known amount of ^{9}Be added and the known fraction of Be extracted from the swimming pool, we can directly calculate the total number of ^{10}Be atoms and the average rate of deposition during the year. The result is 0.055 ± 0.008 atoms cm^{-2} s^{-1}. The error comes essentially from the uncertainty on the measured $^{10}Be/^{9}Be$ ratio, and on the measured fraction of ^{9}Be recovered from the swimming pool.

To estimate an average global deposition rate, we first adjust the above value to the yearly average rainfall for latitude ~50° N. This correction is based on the assumption that the deposition of ^{10}Be at a given latitude will be approximately proportional to the quantity of precipitation[7]. As the average rainfall for 50° N is estimated[8] to be 75 cm compared with a value of 66.7 cm at Orsay during our measurement period, the adjusted average deposition rate at 50° N is 0.062 atoms cm^{-2} s^{-1}. We next apply a factor for the variation of deposition as a function of latitude as given in Fig. 22 of ref. 7. The final result gives a global average value of 0.042 atoms cm^{-2} s^{-1}.

It is difficult to evaluate the uncertainty in the two correction factors which transformed our basic data into an average global rate. However, we believe that our average global rate is the best estimate of the ^{10}Be production rate currently available, and that it is significantly larger than the estimates referred to earlier. It would be interesting to consider the possible sources of error in the earlier estimates. Note that in the case of estimates based on marine sediments[4,5], relatively few samples were involved, and these tended to be biased towards slow accumulation rate areas (to maximise the concentration of ^{10}Be, thus giving adequate activities for the classical counting technique). It is uncertain whether such sediments can be considered representative of the entire ocean floor. In fact, Tanaka and Inoue[9] have measured in several sediment cores ^{10}Be deposition rates comparable to those deduced above. In a rapidly (1.13 cm per 10^3 yr) accumulating sediment at 45° N, we have measured a ^{10}Be deposition rate of 0.10 atoms cm^{-2} s^{-1} (ref. 10). Thus, when a larger and more representative series of sediment results is available, it may well be consistent with our above estimate. Of course, one must also remember that sediment results refer to an integrated production rate over a considerable (and variable) period of time in history, when all the parameters influencing the production (geomagnetic field, solar modulation, primary cosmic ray intensity) may not have been identical to the present.

With regard to estimates based on cosmic ray interaction rates (ref. 4 and F. Guichard, unpublished work) there is a basic problem in choosing appropriate cross-sections. The most important particles in cosmogenic nuclide production in the atmosphere are neutrons. As very few high-energy neutron induced cross-sections are available, values based on proton-induced reactions are usually substituted. As we have pointed out[11], in the particular case of ^{10}Be formed from ^{16}O and ^{14}N, it is quite possible that the neutron induced reactions have larger cross-sections than those induced by protons. We therefore would not be surprised if calculations based on proton cross-sections were underestimated.

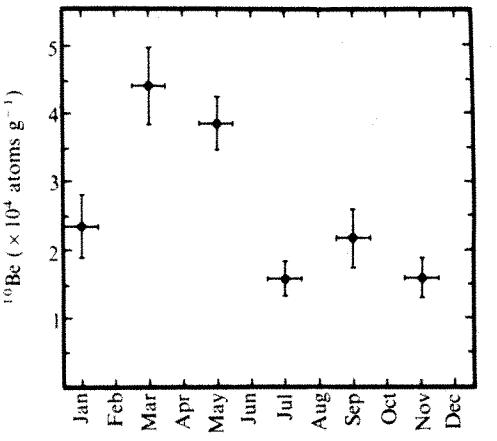


Fig. 1 ^{10}Be concentration in monthly precipitation samples collected in France during 1966.

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In addition to the above determination of the average ^{10}Be deposition rate, we have begun a series of measurements on monthly precipitation samples collected over a 10-yr interval by the Commissariat à l'Energie Atomique (France). Our studies are designed to look for seasonal variations, and eventually, effects due to varying solar modulation. The samples, (30–150 l) were collected at Paris, Orsay, Verdun and Bordeaux using plastic funnels having an area of 0.57 m^2. The precipitation was acidified and reduced to a volume of 15 ml by evaporation. After γ-ray analysis, the samples from the four locations were combined and stored in plastic bottles. We added 4 mg of ^{9}Be to each of these combined samples, which were treated by the solvent extraction process, made into pellets, and measured on the cyclotron as described above.

The results for six of these samples, collected in 1966, are shown in Fig. 1. There is a sharp increase in the ^{10}Be deposition rate during the spring. This is not unexpected, as it corresponds to the period in the year when there is a major transfer of stratospheric air (where ~70% of the ^{10}Be is formed[7]) into the troposphere[12]. It is interesting to consider the possibility that such a seasonal variation might provide a technique for determining accumulation rates in polar ice cores. Our present sensitivity with the cyclotron (~10^7 atoms) has the potential for measuring monthly time resolution with currently available ice cores, and we are preparing a series of samples to test this possibility. However, note that the variations are expected to be smaller in polar regions because the predicted stratospheric component is much less at high latitudes[7].

Finally, while we do not yet have a full year's data for 1966, we can compare the average ^{10}Be concentration in precipitation for the six months analysed (2.33×10^4 atoms g^{-1}) with that for the 1978–79 period (calculated from measured ^{10}Be deposition and the known precipitation rate during that period to be 2.49×10^4 atoms g^{-1}). The agreement is sufficiently good to give us confidence that this is indeed a fairly typical value for France. For a more precise global average deposition rate, similar measurements are needed at several different latitudes.

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Unified yes, renormalised maybe

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Several recent reports[1–3] have suggested that recent neutrino and polarised electron scattering experiments are not particular tests of the 'standard theory' of weak interactions, but only establish the strength and form of the weak neutral current

$$J_\lambda^w = J_\lambda^3 - sin^2\theta_w J_\lambda^{em} \qquad (1)$$

appearing in the effective weak lagrangian

$$\mathscr{L}^{eff} = \frac{4G}{\sqrt{2}}[J_\lambda^w J_\lambda^w + J_\lambda^+ J_\lambda^- + J_\lambda^- J_\lambda^+] \qquad (2)$$

along with the classical charged-current ($J^\pm$) interactions. Whether or not weak vector mesons exist, the current experiments confirm the existence of an effective lagrangian (2) containing a weak neutral current (1) with the one parameter $\sin^2\theta_w$. This letter shows that this already establishes some unification of electron and weak interactions, so that the question is no longer "Is unification really true?"[3], but rather "Is the theory $SU_2\times U_1$ symmetric and renormalisable?" It also shows that, although weak charged currents must show structure[1] below 160 GeV, the weak neutral currents can be structureless, that is $m_z = \infty$ is compatible with the observed current (1).

The first gauge theory of chiral weak interactions[5] had $\sin^2\theta_w = 0$ so that $\mathscr{L}^{eff} = (4G/\sqrt{2})\mathbf{J}\cdot\mathbf{J}$ was SU_2(w) symmetric. This theory ignored electromagnetism and predicted for the first time weak neutral currents of the same strength and form as the weak charged currents. (While this theory was a local gauge theory involving a triplet of vector mesons w$^\pm$ and w^0, the global SU_2(w) suffices for present purposes.) Glashow[4] coupled in electromagnetism, extending the theory to $SU_2\times U_1$, and first proposed the form (1). The history of neutral currents and the local gauge theory of weak interactions after ref. 5 is documented in refs 6, 7. The suppression of strageness-changing neutral currents[8] and the proof of renormalisability[9–11] were most important. Bludman's and Glashow's ideals were early emphasised by Gell-Mann[12] and formed the basis for his algebra of currents and charges. Bjorken has shown[3] that these $\sin^2\theta_w J_\lambda^{em}$ terms in the weak neutral current originate in any large electromagnetic braking of the SU_2(w) invariance such as an intrinsic neutrino form factor much larger than that induced by charged currents[13]. This symmetry-breaking term arises in any theory incorporating γ–w$_0$ mixing[2], but does not require the existence of weak vector mesons and certainly not the particular (Higgs) mechanism for symmetry breaking of the 'standard' theory.

The author was one of the first[14] and most consistent proponents of local gauge theories and to appreciate the theoretical advantages of a renormalisable theory of weak interactions. Renormalisability makes it possible to consider higher order weak processes like the $K_L - K_S$ mass difference and radiative corrections to β- and μ-decay. Certainly the suggestion[15,16] of such a renormalisable theory, together with the development of neutrino accelerator facilities, helped stimulate the discovery in 1973 of the predicted[5] neutral currents. Nevertheless, the present experiments confirm only the strength and form of the proposed neutral currents[4,5]; experiments searching for W$^\mp$, Z^0 and Higgs mesons are needed to test the standard theory[15,16] of weak interactions. At present, spontaneous symmetry-breaking by Higgs mesons apparently introduces as many problems as it solves, and a future dynamical theory may not involve the present theory of the Higgs mechanism at all.

Indeed, Bjorken shows[1] that the charged-current scattering amplitude must be of the form

$$G(q^2) = G(1 + \frac{q^2}{\mu_w^2} + \ldots)$$

with μ_w^{-2} a charged radius bounded from below or μ_w^2 a mean mass bounded from above by

$$\mu_w \leq \frac{e}{(4G\sqrt{2})^{1/2}} \frac{(1-Z_3)^{1/2}}{\sin^2\theta_w} = (37.4\ \text{GeV}) \frac{\sin\theta_1}{\sin^2\theta_w} \qquad (3)$$

Here $\sin^2\theta_w$ is defined by the neutrino charged-radius spectral function and Z_3 is the charge renormalisation induced by intermediate states ('weak quanta') dominating the weak forces. Since $0 \leq Z_3 \leq 1$, we write $Z_3 \equiv \cos^2\theta_1$. With $\sin^2\theta_w \approx 0.23 \pm 0.02$, equation (3) shows that charge current weak inter-

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actions must show structure somewhere below 160 GeV, whether or not intermediate vector mesons exist. If $W^{\pm}$ mesons exist, equation (3) becomes an upper limit on their mass, $\mu_w = (160\ \text{GeV})\sin\theta_1 \leq 160$ GeV. We now show that there is a lower limit (equation (6)) on m_z, the mass of the Z^0. Following Hung and Sakuri[2], consider a phenomenological $\gamma - w_0$ mixing

$$\mathscr{L}^{\text{mix}} = -\frac{\lambda}{4}(\tilde{F}_{\mu\nu}w^0{}_{\mu\nu} + w^0_{\mu\nu}\tilde{F}_{\mu\nu}), \tag{4}$$

which may be a manifestation of a large intrinsic neutrino charge form factor or may originate in quark or lepton vacuum polarisation loops. $\tilde{F}_{\mu\nu}$ is the original unmixed massless photon field. Since the interaction (equation (4)) is electromagnetically gauge invariant, the photon remains massless after mixing, but the W^0 mass is raised to

$$m_z^2 = \frac{m_w^2}{1-\lambda^2}$$

As this requires $\lambda^2 \leq 1$ we can write $\lambda^2 \equiv \sin^2\theta_2$. After mixing, the $\gamma - W_0$ part of the effective lagrangian is

$$\mathscr{L}^{\text{eff}} = \frac{1}{2}\left\{e^2 J^{em}_\lambda \frac{1}{q^2} J^{em}_\lambda + \frac{g^2}{m_w^2} J^w_\lambda \frac{m_z^2}{q^2+m_z^2} J^w_\lambda\right\} \tag{15}$$

where J^w_λ is given by equation (1) with

$$\sin^2\theta_w \equiv \frac{e}{g}\lambda \equiv \frac{e}{g}\sin\theta_2$$

Like Bjorken's, this effective lagrangian depends on two parameters, $\sin^2\theta_w$ and $\sin\theta_1$ or $\sin\theta_2$. (The original $SU_2(w)$ theory predicting neutral currents of universal coupling strength G is recovered if $e/g = 0$ or $\lambda = 0$.)

In terms of θ_2,

$$m_z = \frac{m_w}{\cos\theta_2} \tag{6}$$

$$m_w = \frac{g}{(4G\sqrt{2})^{1/2}} = (37.4\ \text{GeV})\frac{g}{e} = (160\ \text{GeV})\sin\theta_2 \tag{7}$$

While $W^{\pm}$ vector mesons, or at least Bjorken's 'virtual quanta', must exist below 160 GeV, Z^0 could be infinite in mass. In such a case, there would be only two charged vector mesons of mass $m_w = 160$ GeV, and the neutral electro-weak interactions would retain their low energy form at all energies: electromagnetic and weak neutral interactions would be simply of infinite and zero range respectively.

The present generation of experiments establishes the existence of the neutral current (1) with $\sin^2\theta_w \approx 0.23 \pm 0.02$ and $m_w > 30$ GeV. Since this makes $g/e > 0.8$ or the mixing parameter $\lambda \equiv \sin\theta_2 > 0.2$, these experiments demonstrate large electromagnetic breaking[4] of the original[5] $SU_2(w)$ symmetry, or a particle mixing theory that is unified on the tree level of approximation. (The magnitude observed for $\sin^2\theta_w$ may suggest the importance of higher order effects, which would require a renormaliseable theory for their calculation.)

At $q^2 \ll m_z^2$ the effective lagrangian (5) reproduces the ordinary electromagnetic interactions plus the observed weak currents of universal strength $g^2/m_w^2 = 4G\sqrt{2}$. At $q^2 \gg m_z^{22}$

$$\mathscr{L}^{\text{eff}} \approx \frac{1}{2\cos^2\theta_2}\left\{e^2 J^Y J^Y + (e^2+g^2-2eg\sin\theta_2)J^3J^3 + 2e(e-g\sin\theta_2)J^3_\lambda J^Y_\lambda\right\} \tag{8}$$

Hung and Sakurai[2] show the special choice of mixing parameter

$$\lambda = e/g \tag{9}$$

or $\sin\theta_2 = \sin\theta_w$ makes the $\gamma - w_0$ theory asymptotically $SU_2 \times U_1$ symmetric and tree unitary[17–20]. It gives the $w^{\pm}$ vector mesons gyromagnetic ratio $g = 2^2$ and predicts

$$m_w = \frac{37.4\ \text{GeV}}{\sin\theta_w} = 80\ \text{GeV} \tag{10}$$

$$m_z = \frac{m_w}{\cos\theta_w} = 90\ \text{GeV} \tag{11}$$

Whether equations (9)–(11) turn out to be satisfied or not, present experiments establish unification in the tree level of approximation. Neither the two-parameter theory we are proposing nor the standard $SU_2 \times U_1$ theory is yet unified in the sense of containing one coupling constant in a simple group structure. The condition (9), which Hung and Sakurai called the 'unification condition', might therefore more properly be called the 'renormaliseability condition': it guarantees good high-energy behaviour.

The mass relations (10), (11) are those derived in the 'standard theory' from a local $SU_2 \times U_1$ renormaliseable theory with symmetry spontaneously broken by the simplest Higgs mechanism. Future experiments may indeed demonstrate the existence of $W^{\pm}$ and Z^0 mesons of masses (10), (11) and even possibly Higgs scalar mesons. Until then, however, we can only say that present experiments prove the existence of weak neutral currents of the strength originally predicted[5], with strong electromagnetic breaking[4] of the original $SU_2(w)$ symmetry and leave open the possibility $m_w = 160$ GeV, $m_z = \infty$.

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Electrocatalytic oxygen evolution on reactively sputtered electrochromic iridium oxide films

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Oxygen evolution reactions[1] are critical to the development of efficient energy conversion and energy storage devices. For example, power losses at the oxygen electrodes of commercial water electrolysers[2] and H_2–O_2 fuel cells limit their efficiencies to values well below those expected theoretically. The power loss of these devices is ≥15% even at low current densities, and much higher in practical operating conditions. We report here the preparation and the oxygen evolution reaction (OER) electrocatalytic properties of iridium oxide films deposited by reactively sputtering iridium in a humidified oxygen discharge. We find that these sputtered iridium oxide films (SIROFs) have catalytic properties far superior to the best known catalysts for oxygen evolution in acidic aqueous electrolytes at room temperature.

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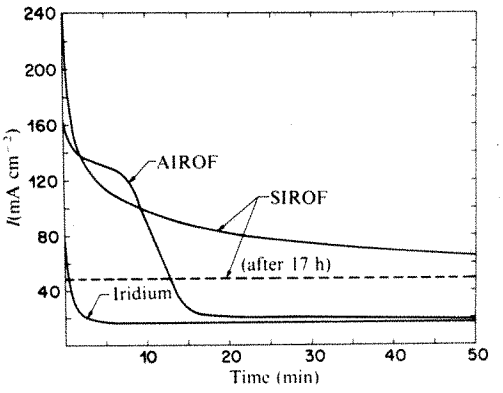


Fig. 1 Oxygen evolution reaction (OER) currents at 1.85 V against RHE as a function of time for anodic iridium oxide films (AIROF), sputtered iridium oxide films (SIROF), and for pure iridium in 0.5 M H_2SO_4 at 25 °C. Despite their high initial current, AIROFs dissolve in the acidic solution so that after ~20 min their OER current is about the same as for bare iridium. In contrast, SIROFs are stable. The dashed line shows the current of SIROFs in the steady state.

Few studies have been aimed at establishing the factors affecting electrocatalysis of O_2, so that we lack the information necessary for a theoretical approach to the search for better O_2 electrocatalysts. Semi-empirically, it has become accepted[1] that a good OER catalyst must have a relatively high electronic conductivity and a high affinity for adsorbed OH^- intermediates. The electrode must not corrode; hence much electrocatalysis research has been restricted to the noble metals and their alloys. A number of recent studies[3-6], however, have pointed out that the link among different electrodes known to be good electrocatalysts for OER (for example, Ir, Ru, Ni) seems to be a relatively thick oxide film which is present on their surfaces. This thick oxide film is clearly different from the more common thin oxide film found on the surface of many metals. In fact these thin films are known to inhibit the rate of many chemical reactions, including the OER. This is why much research ostensibly aimed at solving the problem of electrocatalysis focuses on surfaces devoid of oxides, that is, 'clean' surfaces. In contrast, for 'real' catalysts, thick oxide films (≥1,000 Å) bring about an improved OER.

Experiments show that these thick oxide films display unusual electrochromic effects. Most remarkable is the electrochromism[7-10] of anodic iridium oxide films (AIROFs). Colour-switching times are comparable to those of liquid crystals. AIROF electrochromism takes place at ~1 V (RHE) in 0.5 M H_2SO_4, and is thus a precursor of the OER which occurs at ~1.5 V (RHE). Although the relationship between electrochromism and OER catalysis is not yet completely understood, it is possible that a higher electronic conductivity and increased OH^- content in the coloured state favours the OER. In fact, AIROFs show[3-6,11] good electrocatalytic activity for the OER. A comparison[5] with the performance of the bare iridium electrode indicated that currents obtained for AIROFs on Ir were larger than currents obtained at the same potential on bare Ir.

Although AIROFs have higher OER rates than Ir, they dissolve at significant rates at low voltages (1.4–1.6 V against RHE), and very rapidly at voltages above 1.6 (RHE) in acidic solutions, rendering them of no use as practical catalysts. This initially high catalytic activity and subsequent degradation of AIROFs is illustrated in Fig. 1. After ~20 min the AIROF is totally dissolved and the exposed surface is bare iridium. Thus, in AIROFs, there seems to be a competition between catalytic activity and stability. The high catalytic activity and instability of AIROFs is consistent with the existence of metastable states of intermediate oxidation.

In an attempt to stabilise these metastable states in iridium oxide we reactively sputtered iridium in a humidified oxygen discharge. In the sputter deposition of a thin film, highly energetic species in the vapour phase impinge onto a cold substrate. the rapid quenching of the deposited material tends to stabilise structures that would normally be unstable at room temperature.

SIROFs were deposited on a variety of substrates using an iridium target in a Cooke model C-70-6-4B rf sputtering system. The substrates were washed in a detergent bath, rinsed in distilled water, ultrasonically cleaned in isopropyl alcohol, and vapour-degreased with isopropyl alcohol. They were then placed on a water-cooled J-arm substrate table in the sputtering chamber, which was subsequently evacuated to a pressure of $\sim 2 \times 10^{-7}$ torr. Oxygen at a pressure of 17 p.s.i. saturated with water at room temperature, was admitted into the chamber to a pressure of ~30-μmHg. After a few minutes, the plasma was ignited and the pressure was reduced to ~8 μmHg. Deposition times were ~1 h, typically at an r.f. power of 75 W to a 2″-diameter iridium target corresponding to a target d.c. bias of ~700 V. The metal substrate did not affect the SIROF composition; for example, in the case of tantalum substrates, Auger measurements showed no evidence for any Ta at or near the SIROF surface.

We show elsewhere[12] that the SIROFs have many of the desirable electrochromic properties of AIROFs, including the fast electrochromic response times. The voltammograms for SIROFs are similar to those for AIROFs but differ in detail. In particular, the electrochromic peak for the SIROF occurs at ~0.80 V (RHE) rather than at ~0.95 V (RHE). Both voltammograms are highly symmetric, resulting in excellent stability when operated in the electrochromic range of potentials. We show here that SIROFs (in contrast to AIROFs) are also stable when operated as catalysts in the OER region of potentials, and exhibit a much higher catalytic activity for the OER than bare iridium. Long-term stability tests have not been conducted yet. However, as no degradation has been obtained after ~20 h of operation, SIROFs are at least 2 orders of magnitude more stable than AIROFs.

Figure 1 shows the current of OER for SIROF, AIROF and Ir at 1.85 V (RHE). All currents decay with time until they reach a steady state value (after a few minutes for iridium and after a few hours for SIROF). As noted, AIROFs dissolve rapidly (~20 min). The dashed line shows the steady-state current for SIROFs recorded after ~17 h. The steady-state current is ~3 times as large as the current for iridium, hitherto the best catalyst for OER in acidic solutions.

Tafel plots for OER on SIROFs can be obtained from the steady-state currents recorded after ~17 h at various potentials with no prepolarisation (Fig. 2). Tafel plots for AIROFs have been reported[5]. These plots, however, are practically meaningless as a measure of the AIROF activity because: (1) the currents were arbitrarily recorded 10 min after the initial application of

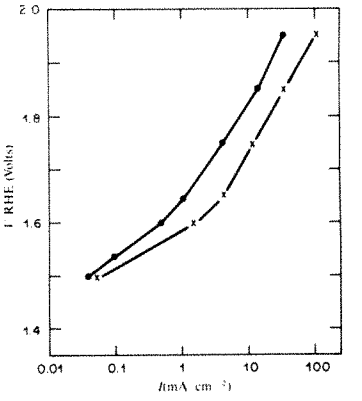


Fig. 2 Tafel slopes for Ir (●) and for SIROFs (×) in 0.5 M H_2SO_4. The data are obtained from currents recorded in the steady state (after ~17 h) with no prepolarisation. Temperature, 25 °C.

the potential to the AIROF electrode; and (2) AIROFs corrode so that the currents recorded are not OER currents but include dissolution currents. At high voltages the slope is essentially equal to that of Ir and other precious metals[13]. An independent measure of the catalytic activity of the material can be obtained by plotting the initial current, after subtracting out the capacitive component. Although the details of this analysis will be reported elsewhere (G.B., L.M.S., J.L.S. and W.C.D-S., in preparation), both steady state and transient measurements clearly indicate the superior catalytic activity of SIROFs.

In general, oxygen evolved on a metal electrode reacts with the metal itself to form an oxide. Thus, the potential at which oxygen is free to leave the electrode is determined by the potential of the metal/metal-oxide couple, or if there is more than one oxide, by the potential of the lower-metal-oxide/higher-metal-oxide couple. Empirically, it has been found[1] that the lower these potentials, the higher the catalytic activity.

Our results confirm this trend. In fact, for AIROFs and SIROFs, the lower-metal-oxide/higher-metal-oxide potential coincides with the electrochromic peak of the voltammogram. As this peak occurs at ~0.80 V (RHE) in SIROFs and at ~0.95 V (RHE) in AIROFs, the higher catalytic activity of the SIROF is consistent with the lower potential of its intermediate oxidation states. Moreover, it is likely that the reactive sputtering procedure 'freezes in' these metastable states of intermediate oxidation, resulting in high stability. Thus, in SIROFs, unlike in AIROFs, no trade-off exists between catalytic activity and stability.

We have shown that stable and efficient electrocatalysts for the oxygen electrode can be fabricated by reactively sputtering iridium in a humidified ambient atmosphere and depositing the sputtered films on substrates of non-precious metals such as tantalum. Also the catalytic activity of the SIROFs is better than that of pure iridium, and this material is stable against dissolution in acidic solutions. As this comparison is based on geometric areas, the 'intrinsic' catalytic activity of SIROFs is still unknown. Measurements of the real SIROF area are in progress. Preliminary results (G.B., L.M.S., J.L.S. and W.C.D-S., in preparation) in alkaline solutions show that steady-state OER currents for SIROFs far exceed the values obtained with the preferred catalysts for alkaline electrolysers[14–18]. For example, whereas $NiCo_2O_4$, at 1.69 V against RHE has an OER current[19] of '50 mA cm^{-2} at room temperature, SIROFs have OER currents in excess of 1 A cm^{-2} after 17 h of operation (G.B., L.M.S., J.L.S. and W.C.D-S., in preparation). Thus, although obviously not optimised, SIROFs seem to be very promising OER catalysts.

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Hydrogen evolution from water using solid carbon and light energy

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Using solar energy to decompose water and produce hydrogen for use in hydrogen energy systems has attracted much attention. Some semiconductors (such as TiO_2 and $SrTiO_3$) can decompose water to produce hydrogen and oxygen using the energy[1] of light and the decomposition of water using powdered semiconductors has been reported[2,3]. One problem with powdered semiconductors is that when H_2O decomposes into H_2 and O_2 in one cell an explosive gas is produced. Furthermore, although Schrauzer and Guth reported[2] that traces of H_2 and O_2 were detected after the decomposition of water gas on TiO_2, the reverse reaction will increase simultaneously as product is evolved. Hydrogen evolution stopped after a few hours. Hence the hydrogen or oxygen produced should be converted to more stable gaseous chemical species. The use of solid carbon is an interesting problem in relation to coal gasification and we have now examined the photocatalytic decomposition of water on TiO_2 using solid carbon. We found that reactions (1) and (2)

$$2H_2O(g) + C(s) \xrightarrow[TiO_2,\ RuO_2]{h\nu,\ RT\sim 80\,^\circ C} 2H_2(g) + CO_2(g) \qquad (1)$$

$$\Delta G^\circ = +63\,kJ\,mol^{-1}$$

$$H_2O(g) + C(s) \xrightarrow[TiO_2,\ (RuO_2)]{h\nu,\ RT\sim 80\,^\circ C} H_2(g) + CO(g) \qquad (2)$$

$$\Delta G^\circ = +92\,kJ\,mol^{-1}$$

$$\xrightarrow{heat,\ 1{,}000\,^\circ C} \text{Thermal water gas reaction}$$

occur producing hydrogen gas from water vapour and solid carbon when mixed powders of TiO_2, RuO_2 and active carbon exposed to water vapour at room temperature ~80 °C are illuminated. The free energies are increased by 63 and 92 kJ mol^{-1} respectively, utilising light energy.

In our experiments 30 mg of TiO_2 (Katayama Kagaku), 10 mg of RuO_2 (Rare Metallic Co.) and 3 mg of active carbon (Katayama Kagaku) were mixed and rubbed in an agate mortar, and spread inside a Pyrex glass bulb (20 cm^2 sample area). The cell and its side arm containing liquid water were then evacuated and illuminated for 5 h in water vapour by a 500 W high-pressure mercury lamp. Any light having a wavelength longer than 320 nm can pass through a Pyrex glass bulb. The gas evolved was trapped at −197 and −78 °C, and was analysed by a mass spectrometer. The gas which was not trapped at liquid N_2 temperature was mainly hydrogen (0.2 torr in 350 ml vessel) and a small amount of carbon monoxide. The gas which was not trapped at −78 °C was mainly carbon dioxide ($H_2 : CO_2 = 1$–1.5) (Fig. 1). When D_2O was used instead of H_2O, D_2 was detected, proving that the evolved hydrogen had been produced from the decomposition of water. Tests were carried out on the systems, TiO_2–H_2O to examine the effect of impurities, and SiO_2–C–H_2O to confirm that without the TiO_2 photocatalyst no appreciable H_2, CO or CO_2 evolved.

Direct reaction of carbon (cokes) with water using heat energy is known as the water gas reaction (2) which takes place at temperatures above 1,000 °C. In our photocatalytic system, however, the reaction with water proceeds at lower temperatures, using carbon as the raw material to decompose water and produce hydrogen. This indicates that oxygen (atom or molecule) produced on the surface of the photocatalyst (TiO_2–RuO_2) has a strong oxidising effect on the carbon.

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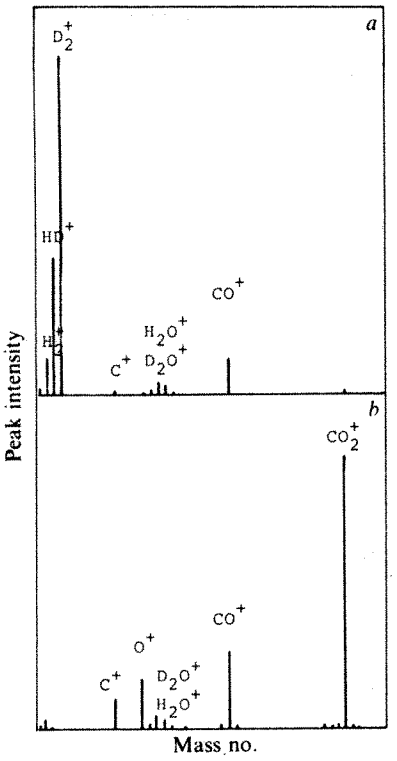


Fig. 1 Mass spectra of the photoproduced gases on TiO_2–RuO_2–C–D_2O (90%). *a*, Fraction not trapped at −197 °C: D_2, H_2 and small amount of CO; *b*, fraction not trapped at −78 °C: mainly CO_2. The C^+, O^+ and CO^+ in *b* are fragments of CO_2^+. H_2O^+ and D_2O^+ are due to background gas and incompleteness of the trapping of water. Pressure of the mass chamber during measurements are 2×10^{-7} torr. 'No hydrogen evolution' means that no D_2 signal was noted when the sample gas was introduced into the mass spectrometer after the photolysis using D_2O. The amount of evolved H_2 and CO depended on how well the catalyst powders were rubbed in an agate mortar. Therefore, the average values obtained after five experiments were considered here.

As TiO_2 powder is regarded as a microcell for the photoredox reaction, water will be oxidised to form oxygen which reacts with carbon to form CO and CO_2, whereas at the reduction site, hydrogen will be formed. Carbon by its own oxidation acts as a reducing reagent. RuO_2 is one of the best electrode materials for oxygen evolution with the lowest overpotential[4]. Hence it may act as the oxidation site at which carbon forms CO and CO_2. In fact, CO_2 formation was suppressed when the RuO_2 catalyst was not added to the system. Only traces of hydrogen molecule could be detected when oxidised TiO_2 photocatalyst alone was used. The addition of RuO_2 and solid carbon markedly increased the hydrogen evolution. Although absolute quantum yield of the reaction has not yet been obtained, the relative quantum efficiency of H_2 evolution in the TiO_2–RuO_2, C, H_2O system increased by at least 10^2 times that from the TiO_2–H_2O system we tested. Consequently, although the increased energy per reactions (1) and (2) is less than that of the decomposition of water into H_2 and O_2, the total energy efficiency for the incident light is much larger in our system. We have examined the effect of several kinds of metals as catalyst (Ru, Ni and Pt) with TiO_2. Although the evolved hydrogen is increased ~7–15 times in the first stage of the reaction, this would be due to the oxidation of metal itself by the oxygen produced from H_2O, assisting hydrogen evolution. Hence the system combined with metal–oxide catalyst is catalytically regenerative. This system remained catalytically active for more than 7 days illumination. When the temperature was raised from room temperature to 60–80 °C, the rate of CO and CO_2 formation increased, probably because a lot of energy is required to split the C—C bond of active carbon to form CO or CO_2. Therefore the use of solar energy as a combination of light energy and heat energy will be more useful.

In the photo-driven ammonia synthesis from N_2 and H_2O (ref. 2) and CO_2 reduction by H_2O (refs 5, 6), hydrogen produced from water was used, whereas the oxygen evolved reacted with carbon, and in our system gaseous hydrogen is produced. Note also that during the reaction, hydrocarbons were produced by reactions among photo-products or solid carbon directly.

As the Earth contains a lot of carbon in the form of coal, this method may be a possibility for coal gasification and hydrogen evolution from water, accompanied by storage of solar energy.

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Phosphorylation of adenosine in aqueous solution by electric discharges

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Dehydration reactions involving condensing reagents in aqueous solution[1] have been studied as models of chemical evolution, and one such reagent has been found to be produced in the supposed primitive Earth conditions[2]. We assumed that the dehydration condensation in aqueous solution could occur if the condensing reagents, which appear to be produced by electric discharges in the gas phase, could be carried to the aqueous solution through the recycling of water washing the wall of the vessel. We present here an experimental study of the dehydration condensation between phosphate and adenosine in aqueous solution using a new discharge apparatus (Fig. 1) which simulates prebiotic chemical evolution. The apparatus was designed so that water recycles in a vessel containing a solution at a relatively low temperature (~30 °C), achieved by showering water at 18 °C from above the vessel. This is a simulation of the recycling system of water on the Earth, depending on a large difference in temperature between the ocean surface and the sky.

Four parallel discharges simulating lightening were induced by four neon-sign transformers (Matsushita, 15 kV), each connected to a ring co-electrode at the centre of the vessel and one of four electrodes around it. The gaps between the electrodes were 3–4 cm. Nichrome wire of 1.6 mm diameter was used for the electrodes. In each of the experiments the aqueous solution and the gases were prepared in the 5-litre discharge vessel in the conditions shown in Table 1. Adenosine was purified on a DEAE-Sephadex column to eliminate impurities such as AMP and other nucleotides. The solution was kept at about 30 °C and stirred with the magnetic stirrer.

The pressure in the vessel changed considerably during the discharge. Figure 2 shows this in runs 1 and 7. The patterns of change were similar in runs 1 to 6, and that of run 8 was to that of run 7 for up to 1 week, thereafter decreasing gradually to 16 cm Hg. The initial increases of the pressure in runs 1–6 are probably due to the decomposition of ammonium bicarbonate. Gas chromatography of the gas component in run 5 [Shimazu Model GC-6APTF; column: active carbon 3 mm × 3 m; column temperature 40–200 °C; carrier gas He, 40 ml min^{-1}] showed that about 90% of its nitrogen appeared as molecular nitrogen, and 30–40% of its carbon as carbon monoxide and dioxide. In runs 7 and 8 the pressure decreased suddenly at the start of

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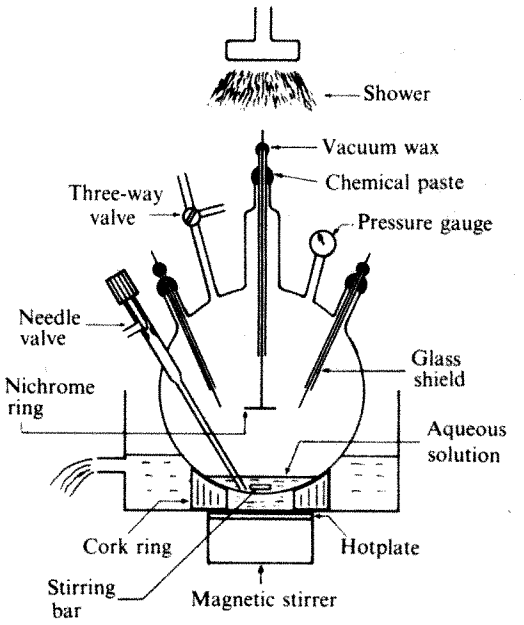


Fig. 1 Five-litre electric discharge apparatus.

discharge, mainly due to consumption of hydrogen. Gas chromatography after a week of discharge showed that the remaining hydrogen constituted only a few per cent of initial level in both cases, but the remainders of the nitrogen and carbon compounds (CO_2+CO) were 87% and 74% (18%+56%) for run 7, respectively, and 85% and 67% (50%+17%) for run 8 (although these results contain errors of several per cent).

After the discharge had taken place for a week, the solution was applied to a DEAE-Sephadex column (1.5×37 cm) and a gradient elution was carried out with 500 ml each of 0.02 M and 0.5 M NH_4HCO_3 (rechromatography was not necessary in runs 7 and 8, because AMP fractions contained only a small quantity of impurities). The fractions expected to contain AMPs were collected, evaporated, and rechromatographed on a Sephadex G-15 column (2.64×65 cm) by elution with 0.1 M NH_4HCO_3 (rechromatography was not necessary in runs 7 and 8, because AMP fractions contained only a small quantity of impurities). The fractions of the main peaks corresponding to AMPs were collected and evaporated to remove NH_4HCO_3, and the residue was subjected to preparative TLC (silica gel) with a solvent system, ethanol ammonia/water, 4:1:1. The extracts from UV-absorbing bands corresponding to 5′-AMP and 2′(3′)-AMP were confirmed to be the expected products on cellulose TLC (solvent system, saturated ammonium sulphate/1M ammonium acetate/isopropyl alcohol, 80:18:2) in which the 2′(3′)-AMP component was separated into two nearly equal UV-absorbing spots corresponding to 2′- and 3′-AMP[3]. Identifications were confirmed by the degradation of the products to adenosine with an application of alkaline phosphatase[4]. Total yields of AMPs are given in Table 1 together with the ratios of the yields of 5′- and 2′(3′)-AMP. The yields are relatively high, considering that the yield of phosporylation is, at most, a few per cent even in the experiments in which a large amount of the condensing agents was used[5], and the very low yields (0.002–0.02%) of the dicyanamide formation in the CH_4–NH_3–H_2O mixture irradiated by electrons[2].

Table 1 Starting conditions and results of analyses after 1 week of electrical discharge

Starting condition	Run no. 1	2	3	4	5	6	7	8
Aqueous solution (100 ml)								
Adenosine (M)	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02
$(NH_4)_2HPO_4$(M)	0.2	0.2	0.2	0.2	0.2	0.2	—	—
KH_2PO_4(M)	—	—	—	—	—	—	0.2	0.2
NH_4HCO_3(M)	1.0	1.0	1.0	1.0	1.0	1.0	—	—
Gas component								
CO_2(cm Hg)	11	21	31	11	11	11	14	14
H_2(cm Hg)	—	—	—	5	—	—	21	14
O_2(cm Hg)	—	—	—	—	5	10	—	—
N_2(cm Hg)	—	—	—	—	—	—	7	7
*p*H	7.70	7.70	7.70	7.70	7.70	7.70	4.80	4.80
*p*H(final)	7.30	7.52	7.30	7.45	7.40	7.40	7.30	7.21
Total-AMP yield (%)*	0.016	0.038	0.029	0.013	0.020	0.010	0.019	0.21
5′-AMP / 2′(3′)-AMP	1.2	1.3	1.3	1.4	1.3	1.1	1.5	1.6

* Total yield values (% adenosine converted to AMPs) contain relative errors of ~±10%.

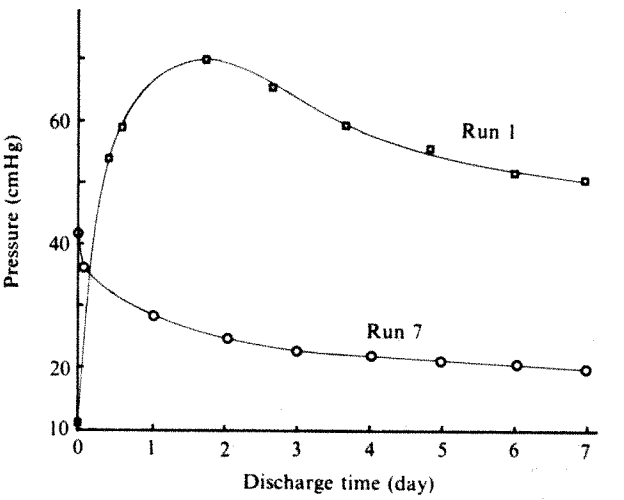


Fig. 2 Pressure change during discharge.

Formation of pyrophosphate was also confirmed in run 8 by detecting phosphate[6] in the fractions corresponding to pyrophosphate after they were boiled for 8 h or treated with pyrophosphatase[7]. The yield of pyrophosphate was estimated to be about 0.01% of the starting phosphate.

A striking result was the finding that the yield of run 8 was one order of magnitude higher than that of run 7. The control experiment in which no electric discharges were applied was also carried out in the same conditions as run 8. Fifty ml of the solution was pumped out after 2 weeks of the incubation. Apart from adenosine, no UV absorbing substances were detected. This indicates that the yield of AMP would be <0.001% which is the limit of the detection. Discharges in run 8 were continued over 1 week to study the dependence of the yield on the

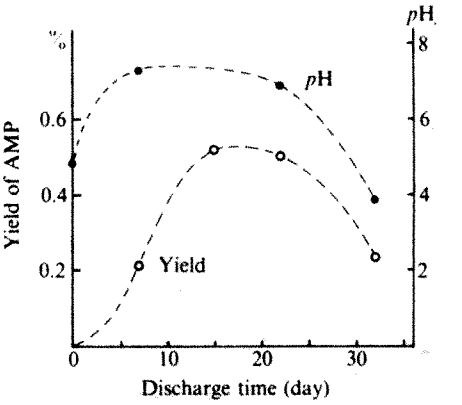


Fig. 3 Dependence of total yield of AMP, and of the *p*H of the solution, on the discharge time in run 8.

discharge time. The findings are shown in Fig. 3, together with measurements of *p*H. The initial increase of *p*H may be due to the formation of ammonia, and the decrease at the latter half to the formation of nitric acid (detected by the method using ferrous sulphate and concentrated sulphuric acid). The remaining solution was pumped out at the end of the run, and the vessel was washed with 10 ml of water. The *p*H of the washing was 0.05. The decrease of the yield at the end is probably due to the rather oxidative composition of the remaining gas, in which nitric acid and other oxidants would have been formed by the discharges. TLC and ion exchange chromatography showed many unknown substances, suggesting that adenosine decomposition took place.

As the results of these runs were dependent on the starting conditions, it seems possible that in certain conditions dehydration condensation could proceed linearly with discharge time. Studies to find such conditions are under way.

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Nascent morphology of polyacetylene

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Acetylene was first polymerised to a linear polymer in 1958[1], but the resulting grey powdery material, insoluble, infusible, and devoid of any interesting electrical properties, remained a scientific curiosity until Ito *et al.*[2] showed that it was possible to polymerise acetylene into a continuous film on the wall of a reactor. This polyacetylene film, $(CH)_x$, a large band gap semiconductor, can be transformed into a highly conducting material with $\sigma_{RT} \sim 1{,}000\ (\Omega\ cm)^{-1}$ (refs 3–12) upon doping with either electron donors or acceptors[13,14]. Although fibrous electron micrographs (EM) of unaligned[2] and partially aligned (stretch elongated) samples having enhanced conductivities have been reported[3], it has been suggested that the observed morphology might be misleading, possibly resulting from a post-polymerisation handling of the film, or even an artefact such as the result of ultrasonication. The aim of this work is to determine the true nascent morphology of polyacetylene. We have polymerised acetylene directly on electron microscope gold grids and examined the polymer with scanning and transmission modes. The ultrathin specimen is entirely fibrous with diameters of ~5–40 nm, with 20-nm fibrils the most abundant. Some films show surface fractures revealing partially or completely aligned fibrils bridging the gaps. Some globular features were also observed suggesting local fusion. Thick films of polyacetylene also have fibrous morphology throughout. The observed structure is consistent with surface area measurements. Therefore, acetylene polymerises directly into fibrils, possibly due to the rigidity of the polymer backbone which is comprised of conjugated double bonds.

Acetylene was polymerised directly onto EM grids (3-mm disks of 300-mesh gold screen). This was accomplished by indium soldering of the grid on to a fine gold wire and hanging it in the reactor above the catalyst solution. The catalyst solution [7 mM $Ti(OBu)_4$, 28 mM $Al(C_2H_5)_3$] was introduced onto the grid as a thin liquid film by shaking. Acetylene gas (total pressure ranging from 10 to 760 torr of acetylene) was admitted for less than 1 min and pumped off immediately. The thickness of the polymer film may be controlled by varying the catalyst concentration, acetylene pressure and the time and temperature of the reaction. The ultrathin $(CH)_x$ specimen thus prepared was washed to remove catalyst residues by repeated pentane distillation. A JEOL 100 CX Temscan electron microscope was used in this work. The four modes of observation were transmission (TEM), electron diffraction (ED), scanning (SEM) and scanning transmission (STEM). The polymerised film was observed by the first two modes; gold-coated films were examined with the other two modes. Gold coating was applied either by sputtering or evaporation with its thickness carefully controlled and kept in the 3–7-nm range.

Figure 1*a* is a STEM image at low magnification (10^4) showing fibrous webs throughout the specimen. At higher magnification of 5×10^4, Fig. 1*b*, *c* shows STEM images of the identical portion of the sample in which voids appear black (and white), respectively, demonstrating that the film is only a few fibrils in thickness. At 10^5 magnification (Fig. 1*d*) individual fibrils were clearly discernible having diameters ranging from ~5 to 40 nm with 20-nm diameter fibrils being the most abundant. BET measurements (nitrogen gas) gave a surface area of $60\ m^2\ g^{-1}$ which corresponds well with the observed fibre morphology.

Interestingly, polymerisations at higher acetylene pressure (760 torr) yield extremely smooth film on the grid even when observed at the highest magnifications. These films, however,

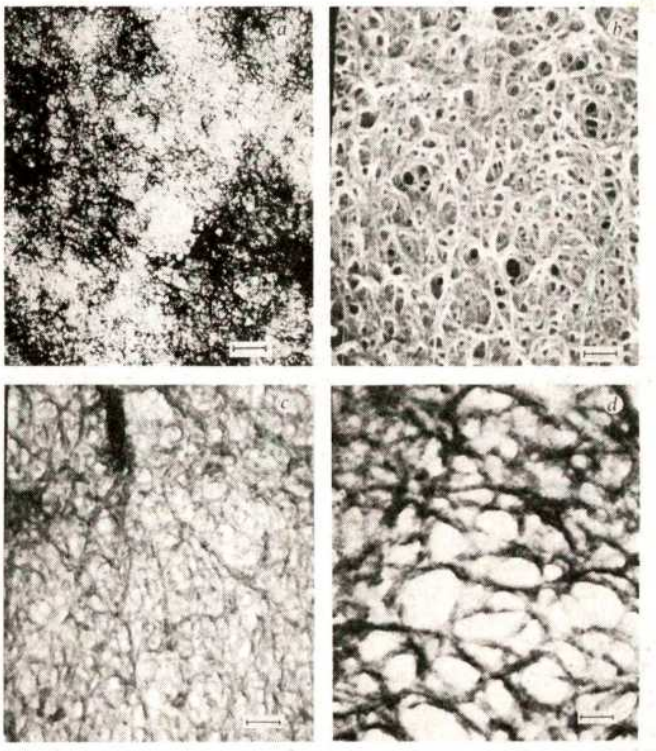


Fig. 1 *a*, Electron micrographs of ultrathin $(CH)_x$ films polymerised directly onto a gold grid at 55 torr of acetylene for 30 s, dried and coated with a 3-nm later of palladium gold by evaporation, ×10,000 in the STEM mode. *b*, Same sample as in *a* at ×50,000 SEM for matched areas. *c*, As for *b* but in STEM mode. *d*, Same sample as in *a* showing 100,000 STEM. Scale bars: *a*, 1 μm; *b*, *c*, 0.2 μm; *d*, 0.1 μm.

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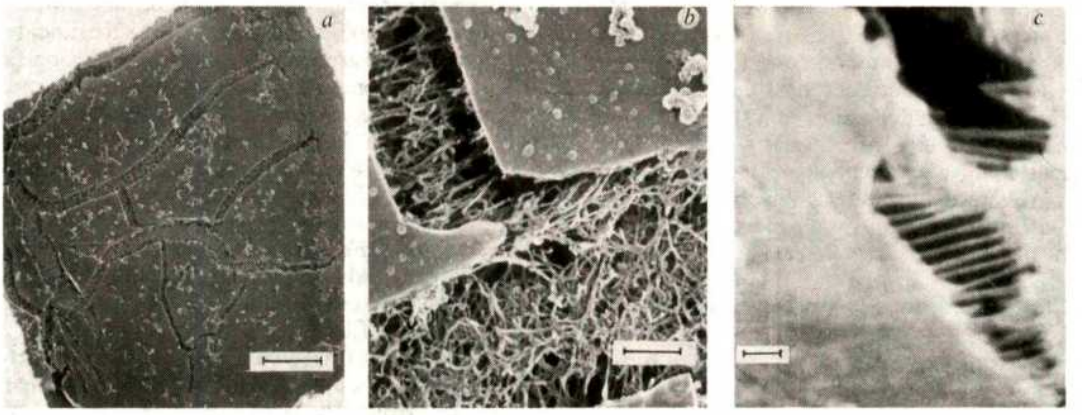


Fig. 2 SEM micrographs of thin $(CH)_x$ films polymerised directly onto a gold grid at 760 torr of acetylene for ~1 min, dried and sputter coated with 3 nm of gold. *a*, ×1,500; *b*, ×15,000; *c*, ×100,000. Scale bars: *a*, 10 μm; *b*, 1 μm; *c*, 0.1 μm.

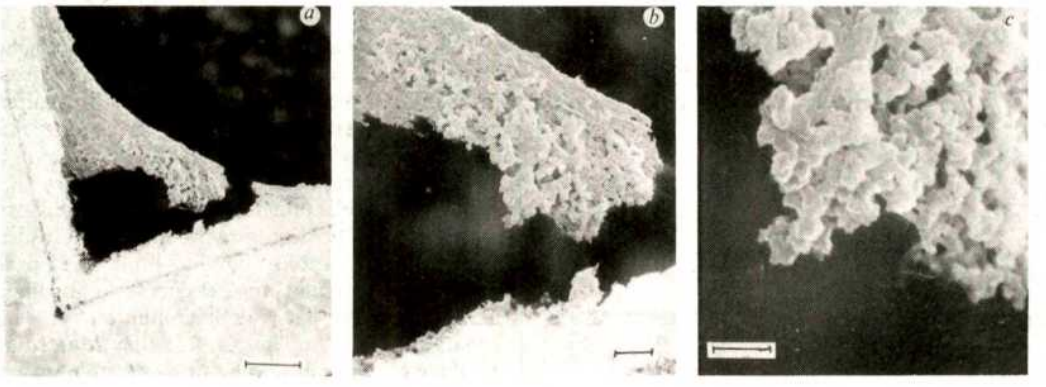


Fig. 3 SEM micrographs of another thin $(CH)_x$ film polymerised as in Fig. 2. *a*, ×3,000; *b*, ×8,000; *c*, ×30,000. Scale bars: *a*, 5 μm; *b*, 1.25 μm; *c*, 0.5 μm.

possess fractures (Fig. 2*a*) in which are found partially aligned fibrils (Fig. 2*b*). Occasional regions contain highly aligned 10-nm diameter fibrils (Fig. 2*c*). These fractures are probably formed during the washing procedure and are due either to the displacement of one diluent (toluene) with another (pentane) or to the thermal contraction occurring when the film was cycled between 78 K and room temperature. One explanation for the smooth film surface is that it consists of layers of $(CH)_x$ polymerised in a substantially aligned state.

There are also globular structures, as shown, with increasing magnification, in Fig. 3. Examination of Fig. 3*a* shows that the globular growth appears to have been separated from a portion of the film as a result of melting contraction. Such local heating is entirely possible as the polymerisation is highly exothermic.

That the above morphology extends to thick continuous films formed by depositing a thin layer of catalyst solution on the wall of a cylindrical reaction vessel and subsequently exposing this layer to an atmosphere of acetylene, with polymerisation extending for several minutes, may be readily demonstrated. Such film has a highly reflecting metal-like surface on the side adjacent to the glass wall of the reactor (Fig. 4*a*) and a duller surface on the opposite side (Fig. 4*b*). The latter has the typical random fibrous morphology discussed above. The shiny side apparently has an identical morphology except that the fibrils are flattened and merged or fused into larger entities. Fusion is a distinct possibility even though one might expect heat transfer to be more efficient at the reactor wall. However, polymerisation is likely to occur very rapidly there because of a high catalyst concentration and lower monomer diffusion limitation.

Continuous $(CH)_x$ film has a gross density of only ~0.4 g cm^{-3} whereas its flotation density is 1.16–1.18. Therefore, the film is highly porous with voids constituting two-thirds of the total volume. When the fibrils are not aligned, the gross morphology of $(CH)_x$ film is somewhat analogous to the cellulose fibrils constituting a sheet of paper.

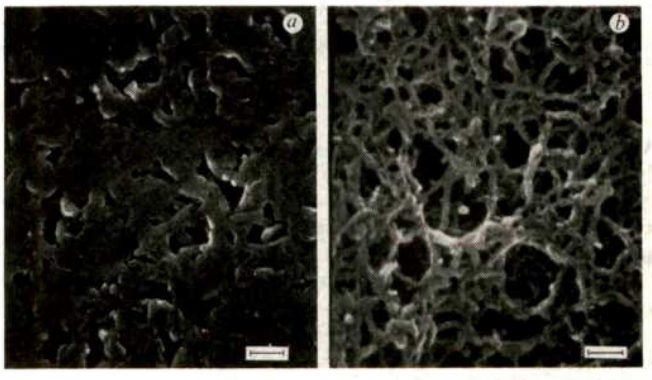


Fig. 4 SEM micrographs at ×50,000 of thick $(CH)_x$ film polymerised on the glass wall of reactor with 760 torr acetylene. *a*, Highly reflecting side of the film facing the wall; *b*, dull side of the film facing away from the wall. Scale bars, 0.2 μm.

Ethylene and propylene polymerised by Ziegler–Natta catalysts have fibrillar, spherical, globular, worm-like, needle-shaped, and helical nascent morphologies depending on the type of catalyst and experimental conditions. Polyethylene fibres of 20–30 nM were obtained with $TiCl_3/AlEt_3$ catalyst[14]. Polypropylene fibrils of 30–50 nM diameter were formed by the

same catalyst[15]. The mechanism suggested was that the polymer chains formed on adjacent active sites of the α-$TiCl_3$ surface were insolubilised together into microfibrils[16]. The present $Ti(OBu)_4/AlEt_3$ catalyst has been described as an homogeneous or an heterogeneous system depending on experimental conditions[17–19]. The fact that uniform fibrillar polyacetylene was produced suggests an heterogeneous catalysis mechanism. Work is in progress to determine the dependence of nascent polyacetylene morphology on catalyst concentrations and polymerisation conditions.

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^{87}Rb–^{87}Sr chronology of the Binda howardite

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Differentiated meteorites have numerous similarities with igneous rocks from the Earth and the Moon. They are of primordial importance in our understanding of magmatic processes in the early Solar System. The howardites constitute a particular class of differentiated meteorite as they are mechanical mixtures[1–7] (polymict breccia) of components from two other classes—eucrites and diogenites. The eucrites are representative of magmatic liquids and the diogenites are the corresponding cumulates[3]. This relationship is supported by oxygen isotopic data[8]. The ^{87}Rb–^{87}Sr chronology of eucrites[9,10] shows that these objects result from a very early igneous activity near the surface of their parent body (<150 Myr after formation of the Solar System). As for howardites, within the same meteorite Kapoeta, basaltic clasts have been dated by the internal Rb–Sr isochron at 4,400 Myr and around 3,500 Myr (refs 11, 12). ^{39}Ar–^{40}Ar dating of these clasts provides evidence for their initial crystallisation 4,500 Myr ago, and for their independent history until the final lithification of Kapoeta, probably as late as 2,200 Myr ago[13]. Due to this complexity their initial age of crystallisation, and in particular, their $^{87}Sr/^{86}Sr$ initial ratio is not precisely known. We report here our investigation of the Binda howardite which seems to have had a simpler history; it is the only one which is a monomict breccia. The component representing the cumulate fraction seems to be in equilibrium with that which represents the eucritic melt[3]. Therefore one might expect to date the howardites formation by dating this meteorite, assuming that they all formed in the same parent body.

Minerals were separated from the meteorites by hand-picking and heavy liquid methods. Our experimental technique is similar to that used for the Lunar 24 basalt analysis[14]. Blanks are 2 ng, 0.004 ng, 0.03 ng for K, Rb and Sr, respectively. (Blank correction was never more than a few per cent.)

The results are given in Table 1, and Fig. 1 shows the conventional Rb–Sr diagram. Although the total rock samples of Binda are between those of Juvinas and Bereba[10], concentrations in K, Rb and Sr are a factor of 2 to 5 lower than in these meteorites. The cumulate fractions are very poor in these elements[7] and from our K–Rb–Sr concentration data, they must represent at least 50% of the Binda howardite. By plotting the data in the $^{87}Rb/^{86}Sr$, $^{87}Sr/^{86}Sr$ diagram (Fig. 1) it may be seen that this object has undergone perturbation. However, all the points except one (PXH3) fall on a straight line within experimental error limits. This line corresponds to an age of 3,450 ± 340 Myr (2σ) with $\lambda = 1.42\ 10^{-11}\ yr^{-1}$ as decay constant for ^{87}Rb.

Heavy liquid can have preferential leaching effects on meteoritic and lunar minerals[15]. This can be understood if we assume that some fine particles become partially dissolved during the separation, because they are not separated from heavy liquids even when these are centrifuged. For achondrites[9,10] and some lunar samples[14] this seems to be the case for the light interstitial K–Rb rich phases, and might significantly affect the age. In the case of Binda, however, we believe that such an effect cannot produce our results. First, we have made a very rapid separation using small quantities of heavy liquids for Binda expecting that the effects increase with time. Second, short-time leaching by heavy liquids generally increases the model age of the leached fractions. Finally, both hand-picked and heavy liquid separates plot on the same straight line. We have also analysed the K, Rb and Sr contents of the liquors after the separations and (unlike ref. 15) they represent <1.3% of the total concentrations in the samples (Table 1). We conclude that the 3,450 ± 340 Myr age of Binda is not an experimental artefact and at least sets an upper limit for the age of the last remobilisation of the strontium but most probably dates this one.

The $^{87}Sr/^{86}Sr$ initial ratio calculated from whole rock assuming an age of 4,470 Myr is 0.69894 ± 0.00004. Similarly, if the hand-picked plagioclase 'Plag H' is assumed to have the same age, this indicates an $^{87}Sr/^{86}Sr$ initial ratio of 0.69894 ± 0.00004. This value is indistinguishable from that for eucrites[10–16]. The small insignificant difference might be due to a non-representative whole rock sample. A small excess of pyroxene in the latter may explain the difference. One can compare this $^{87}Sr/^{86}Sr$ initial ratio to that for the other howardite studied so far: Kapoeta[11,12]. That for Kapoeta is significantly lower in clast C which has an age of 4,440 Myr and $^{87}Sr/^{86}Sr$ initial ratio of 0.69885 ± 0.00005. The Sr initial ratio for clast A is almost as low as that for clast C. We are faced with two alternatives. First that this low initial value is real. In this case the basaltic clasts (A and C) are not related to the magmatism which created Binda and the eucrites. They may come from another part of the same parent body or Kapoeta comes from another parent body with a similar history with a different timing. In any case, Kapoeta contains exotic components like chondritic material[1], and its formation seems to involve many components[17] of various origins. The alternative interpretation is that this low initial ratio is insignificant and results from Kapoeta being a mixture of different components of various ages; a similar interpretation has been discussed for lunar samples[18]. We cannot distinguish between these possibilities at present.

As in eucrites, the ^{87}Rb–^{87}Sr mineral age for Binda is controlled by a light K-rich phase. The internal age might simply relate to this mineral. One can interpret this younger age as a secondary resetting of the ^{87}Rb–^{87}Sr clock by metamorphism or shock, 3,450 Myr ago. This result is similar to the ^{87}Rb–^{87}Sr and ^{39}Ar–^{40}Ar ages around 3,800 and 3,500 Myr of some clasts of Kapoeta[11–13]. It also agrees with the 3,600 Myr ^{39}Ar–^{40}Ar age of Malvern[19]. At the opposite, the ^{39}Ar–^{40}Ar ages of the

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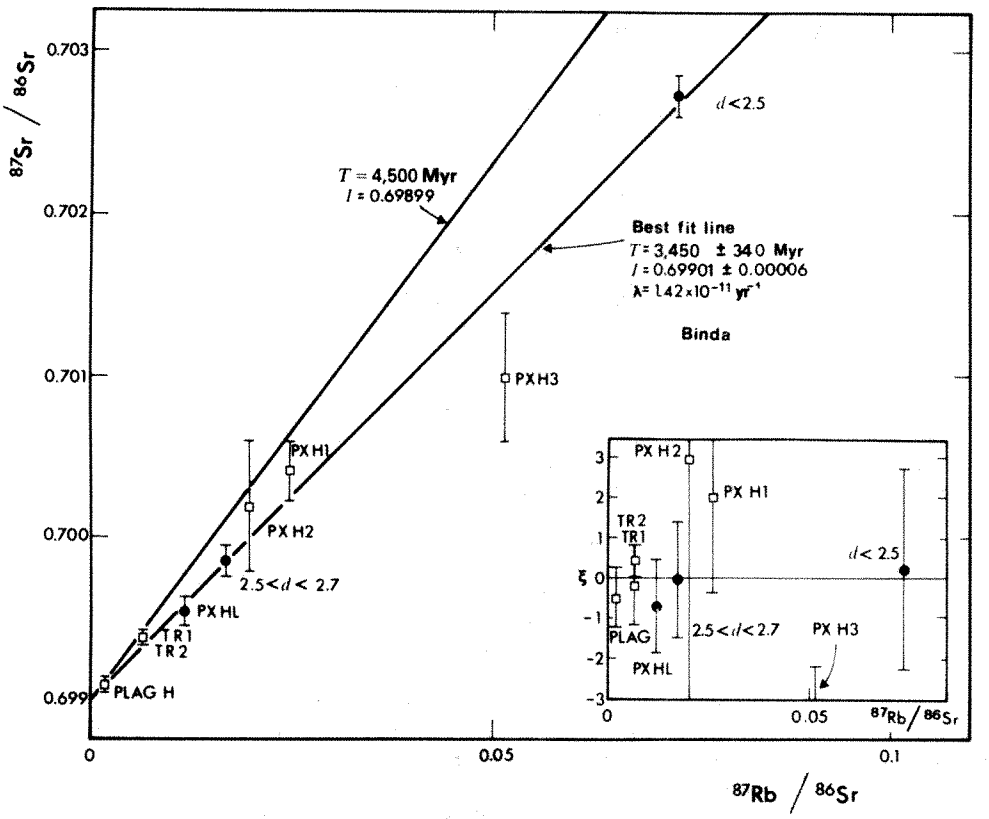


Fig. 1 Rb–Sr evolution diagram for the minerals of the Binda howardite. The decay constant used here for ^{87}Rb is $\lambda = 1.42\,10^{-11}\,yr^{-1}$. TR, total rock; PX, pyroxene; Plag, plagioclase. The inset shows the relative deviation in 10^{-4} units of the experimental points from the best fit line as a function of $^{87}Rb/^{86}Sr$. The reduced χ^2, σ is 0.84 and shows that the points can be fitted to a straight line within experimental errors. The anticorrelation between the errors of slope and intercept is $s = -0.639$ (see ref. 23). ●, Heavy liquids; □, handpicked.

Table 1 Experimental results for Binda

Sample	Weight analysed	K (p.p.m.)	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}Rb/^{86}Sr$*	$^{87}Sr/^{86}Sr$†
Total rock 1	40.0	80.0	0.0925	39.7	0.00643	0.69936 (3)
Total rock 2	23.3	68.7	0.0752	33.63	0.00647	0.69932 (7)
Pyroxene H1‡	10.2	2.94	0.00593	0.690	0.0249	0.70040 (17)
Plagioclase H‡	2.0	267.0	0.0897	135.2	0.00192	0.69907 (5)
Pyroxene H2‡	21.0	3.29	0.00657	0.977	0.0194	0.70019 (44)
Pyroxene HL‡	45.1	13.1	0.0243	6.08	0.01159	0.69954 (8)
Pyroxene H3‡	6.0	6.47	0.0169	0.951	0.0517	0.70099 (40)
$2.5 < d < 2.7$§‖		166.0	0.212	36.6	0.0168	0.69985 (10)
$d < 2.5$§		95.0	0.317	12.50	0.0734	0.70271 (18)
Heavy liquid content§‖		285.0	0.40	2.5	0.46	

* The error on this ratio is 1%.
† Errors are 2σ quoted on the last decimal place.
‡ H, handpicked; HL heavy liquid separates.
§ These data are given in nanograms because the weight is unknown.
‖ The heavy liquid (methylene iodide + acetone) was in contact with 320 mg of powder.

howardites Bununu and Yamato L are higher than 4,200 Myr (ref. 19) and 4,000 Myr (ref. 20) respectively. Similarly ^{39}Ar–^{40}Ar dating of Kapoeta also yields ages ≥4,500 Myr (ref. 13). If all howardites are genetically related, these data show that they probably crystallised 4,500 Myr ago and were later disturbed by one or several large scale secondary events 3,400–3,800 Myr ago which are probably related to impacts. As discussed by Wetherill[22], the absence of a cluster of resetting ages around 4,000 Myr is not a conclusive argument against an inner Solar System wide 'cataclysm' such as determined on the Moon[21]. However, there is a lot of evidence for large-scale impacts in Basaltic Achondrites between 3,900 and 3,500 Myr which are not so far recorded in the lunar rocks actually analysed.

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Allende meteorite—old age but normal isotopic composition of potassium

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The extensive investigation of the Allende meteorite has provided much new information on the early history of our Solar System. The findings include small but significant anomalies of the isotopic composition for several elements which are clearly not due to any of the processes hitherto known to cause such variations (see ref. 1 for review). These anomalies seem to confirm the reality of astrophysical concepts developed since the classical paper of Burbidge *et al.*[2] on the different modes of origin of the nuclei. Evidence has also been found which points to the possible presence in Allende of pre-solar condensates. Jessberger and Dominik[3] have presented data to support an earlier result[4] according to which the ^{40}Ar gas retention ages of some white inclusions from Allende exceed the canonical age of the Solar System. These gas retention ages were obtained by ^{40}Ar–^{39}Ar dating, however, and are therefore based on the assumption that the ^{39}K/^{40}K ratio in the inclusions is identical to that used in the reference standard. In view of the far-reaching implications of finding in meteorites solid matter pre-dating by roughly 500 Myr the condensation of the Solar System we have attempted to verify this assumption. This is especially important because the Allende inclusions contain elements with an anomalous isotopic composition.

We were given (by E. K. Jessberger) two portions of inclusion 18: three chips with a combined weight of 1.9 mg and another aggregate of 16 mg. Although these portions are not true aliquots of a well homogenised sample they are, nevertheless, considered representative of the sample analysed by Jessberger and Dominik.

The small sample was used for pilot experiments. One chip (780 μg) was treated in a small Teflon bomb with a mixture of 5 μl HF (40%) and 3 μl $HClO_4$ (70%) for 5 h at 190 °C. It was fumed to dryness in the Teflon bomb and taken up with 20 μl HF (10%). Part of the solution was spiked with both ^{40}K and ^{42}Ca for the determination of the concentrations of potassium and calcium by isotope dilution, the remainder was used for the potassium analysis. If there had been a residue its distribution among the spiked and the unspiked fraction may have been inhomogeneous.

Although using HF to take up the sample drastically reduced the Ca^+/K^+ ratio in the mass spectrometer, the interference on mass 40 from $^{40}Ca^+$ could not be entirely neglected. Hence, from all subsequent samples with one exception (see below) the potassium was separated by ion exchange. This resulted in very weak signals on the monitor masses 42 and 44 but, because of the high ^{40}Ca/^{42}Ca and ^{40}Ca/^{44}Ca ratios respectively, it caused a large uncertainty in the residual interference on mass 40. This was avoided by adding a ^{42}Ca spike to the potassium after it had been separated on the ion exchange column. (The resulting ^{40}Ca/^{42}Ca ratio itself was measured after termination of the potassium measurement by increasing the temperature of the sample filament until Ca dominated the mass spectrum.) The spike solution contained 10^{-3} g K of normal isotopic composition per gramme of ^{42}Ca.

The remaining two chips from the small sample (1,100 μg) were treated as before (10 μl HF + 4 μl $HClO_4$, 5 h at 180 °C, fuming to dryness, 5 μl each of HF and $HClO_4$, 10 h at 190 °C, fuming to dryness). The residue was taken up with 20 μl of 0.5 M HCl. Part of it was spiked with ^{40}K and ^{42}Ca, the rest (95%) was filtered through a Teflon filter (Millipore FH 0.5 μm) and the filtrate put on a quartz ion-exchange column (3 mm diameter, length 5 cm) filled with Dowex 50 WX 8. Potassium was eluted with 0.9 M $HClO_4$. The eluate (0.63 ml) was spiked with 12 ng of ^{42}Ca, it was evaporated to dryness and dissolved again in 5 μl HF (10%) for the mass spectrometric measurement.

The 16 mg aggregate was decomposed as described above except that larger amounts of acid were used and that the heat treatments in the Teflon bomb were 48 h and 15 h, respectively. After the last fuming to dryness the residue was taken up in 530 μl of 0.9 M $HC10_4$ and centrifuged. About 1% of the supernatant was spiked with ^{40}K and ^{42}Ca, the bulk put first through a large (4mm diameter, length 5 cm) and then a small ion exchange column. In both instances potassium was again eluted with 0.9 M $HClO_4$ and ^{42}Ca was added as before. The undissolved portion of the aggregate, the residue after centrifuging, amounted to <1% of the original 16 mg. It was brought into solution by treating it in the Teflon bomb for 16 h at 190 °C with 5 μl each of H_2SO_4 (96%) and H_3PO_4 (85%). It was analysed by mass spectrometry without any more chemistry.

Only quartz and Teflon were used throughout the experiments; all acids were Merck grade 'suprapur'. Their K-content was determined by isotopic dilution and was found to be for HF (40%) 4 p.p.b. (parts per 10^9); $HClO_4$ (70%) 50 p.p.b.; HCl (4.5 M) 1 p.p.b.; H_2SO_4 (96%) 80 p.p.b.; H_3PO_4 (85%) 140 p.p.b. and H_2O 1 p.p.b. The total blank of dummy runs with

Table 1 Elemental concentrations of K and Ca and isotopic composition of potassium

Sample	(mg)	Run	Detector*	K (p.p.m.)	Ca (%)	^{39}K/^{41}K Max	Min	$(^{41}K/^{40}K)_{corr}$	$I(^{40}Ca)/I(40)$	K-blank (%)
Terrestrial		112	*M*	—	—	14.099	13.899	573.7 ± 7.1	—	—
		112	*F*			13.800	13.727	586.0 ± 10.0		
JD18-SI	0.78	114	*M*	85 ± 20	11.3 ± 1.2	14.405	13.819	562.4 ± 13.6	$\leq 8\times10^{-2}$	≤3
Terrestrial		116	*F*	—	—	13.862	13.541	585.1 ± 3.6	—	—
Terrestrial		131	*M*	—	—	14.329	14.167	572.7 ± 10.8	—	—
		131	*F*			13.935	13.703	585.4 ± 6.2		
Terrestrial		134	*M*	—	—	14.138	14.090	576.9 ± 4.1	—	—
JD18-SII	1.10	135	*M*	21 ± 4	8.3 ± 0.8	14.311	14.066	574.0 ± 5.5	$\leq 1.2\times10^{-4}$	≤43
Terrestrial		141	*M*	—	—	14.304	14.220	575.3 ± 2.1	—	—
JD18-SIII	15.93	142	*M*	114 ± 20	13.0 ± 1.2	14.194	14.100	572.1 ± 3.0	$\leq 2.4\times10^{-4}$	≤0.6
Terrestrial		144	*F*	—	—	13.860	13.807	581.6 ± 4.6	—	—
Terrestrial		146	*M*	—	—	14.306	14.145	568.4 ± 14.4	—	—
JD18-SIII	15.93	147	*M*	114 ± 20	13.0 ± 1.2	14.219	14.160	573.0 ± 6.7	$\leq 0.6\times10^{-4}$	≤0.6
		147	*F*	—	—	13.832	13.712	582.3 ± 2.2		
Terrestrial		148	*F*	—	—	13.902	13.850	585.5 ± 1.5	—	—
JD18-SIIIR		150	*M*	—	—	14.411	14.138	579.5 ± 2.4	$\leq 2\times10^{-3}$	—

The ^{41}K/^{40}K ratios are corrected for mass discrimination only, their errors are ±1 σ (mean standard deviation). Sample SIIIR is the residue from SIII.
* *M*, secondary electron multiplier, *F*, Faraday cup.

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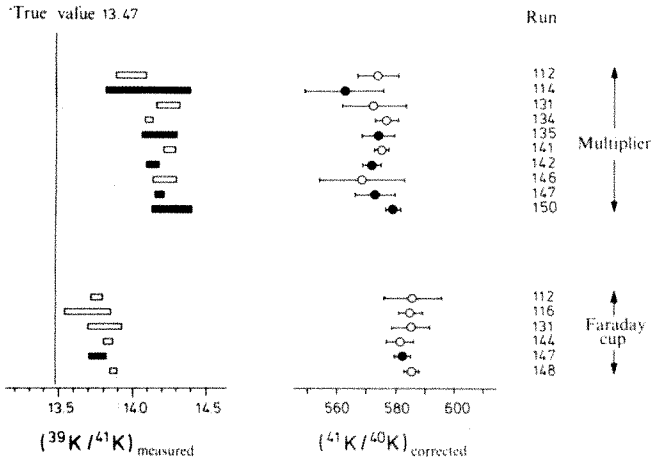


Fig. 1 Measured $^{39}K/^{41}K$ ratios and mass discrimination-corrected $^{41}K/^{40}K$ ratios of terrestrial (open symbols) and Allende (filled symbols) samples. The length of the bars for the measured $^{39}K/^{41}K$ ratios do not indicate the errors of these ratios but rather the shift with time observed during the measurement. The Faraday cup measurements are in complete agreement with the results of Burnett *et al.*[10] and Begemann and Stegmann[7] who, after normalisation to the same $^{39}K/^{41}K$ ratio of 13.47, reported values of 584.89 and 584.87, respectively.

appropriate amounts of acids was ≤2 ng K, passing the solution through the ion-exchange columns increased the value to ≤10 ng.

The mass spectrometric measurements were performed by surface ionisation using double (rhenium) filament sources. Only in the case of the 16 mg sample was the ion current high enough for a Faraday cup, together with a $10^{11}\Omega$ resistor, to be used as a detector. This necessitated a correction for the nonlinear voltage dependence of the resistor; a second order polynomial gave a fit through the experimental data to better than 3×10^{-4} for the whole range of interest (10–65 V). In all other instances a 16-stage secondary electron multiplier (1,500–1,800 V, $10^9\,\Omega$, gain $\approx 2\times10^5$) was used where no such correction was necessary. Data acquisition was by computer-controlled peak jumping using a $5\frac{1}{2}$-digit integrating voltmeter. Details will be published elsewhere[5].

The results are compiled in Table 1. The potassium contents in column 4 are uncorrected for blanks, the last column gives upper limits for the contribution of blank potassium to the total, based on 2 ng K for samples SI and SIIIR and 10 ng for SII and SIII (see above). Owing to the low ion currents in the mass spectrometer a considerable fraction of the total potassium had to be measured. Consequently, a marked shift with time of the isotope ratios was observed. This is indicated by the range of $^{39}K/^{41}K$ ratios in column 7, where the highest values are the initial ones. The $^{41}K/^{40}K$ ratios are corrected for fractionation by adopting as true value $^{39}K/^{41}K = 13.47$ (ref. 6) and assuming the mass discrimination between neighbouring isotopes to be constant. In no case was there any positive evidence for a contribution to mass 40 from $^{40}Ca^+$; the limits which can be set are indicated in column 9.

Figure 1 shows the isotope data of Table 1, together with results for normal terrestrial K as obtained on samples of similar size as the meteoritic ones. The degree of the instrumental fractionation can be seen from the off-set of the measured $^{39}K/^{41}K$ ratios from the Nier value of 13.47. Particularly obvious is the additional discrimination caused by the mass dependent gain for different isotopic species of the multiplier. It also seems that the corrected $^{41}K/^{40}K$ ratios are systematically lower for the multiplier compared with the ones with the Faraday cup as collector. Contributions on mass 40 from species other than ^{40}K can be excluded as was verified by large terrestrial samples. The reason for this discrepancy is not clear. As both the terrestrial and the meteoritic samples are affected, however, there is no potential reason apparent for any misinterpretation as long as both sets of data are considered separately.

We conclude from these data that within the experimental limits of error the isotopic composition of potassium from the Allende inclusion is indistinguishable from that of normal potassium. Neither the $^{39}K/^{41}K$ nor the $^{41}K/^{40}K$ ratio shows any evidence for an anomaly in excess of 1%. As pointed out by Jessberger and Dominik[3], for a sample 4,600 Myr old to yield an apparent ^{40}Ar–^{39}Ar age of 5,100 Myr the ^{40}K would have to be enriched by about 35%. As our upper limit to a possible anomaly falls short of this by more than an order of magnitude the old apparent age of this inclusion cannot be due to an anomalous abundance of ^{40}K.

As one of the assumptions underlying the ^{40}Ar–^{39}Ar method has been verified the old apparent ages of some of the Allende inclusions are more likely to be the true ones. Unfortunately, however, the interpretation is still ambiguous as another prerequisite for obtaining true ages is that all ^{40}Ar from the sample is indeed produced by the *in situ* decay of ^{40}K. We feel that it would be premature to exclude the possibility of the presence in meteoritic matter of extraneous 'unsupported' or 'orphan' ^{40}Ar.

We should re-emphasise that in the inclusion investigated the relative abundance of ^{41}K agrees with the normal value to within ±1%. Thus there is no evidence[7,8] to support Clayton's suggestion[9] that pre-solar grains might contain excess ^{41}K from the *in situ* decay of ^{41}Ca. This result will be particularly important if the ^{40}Ar–^{39}Ar ages should turn out to be the true ages as this would make the inclusions, or part of them, true presolar condensates.

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Age and significance of alluvium in the Windrush valley, Oxfordshire

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The floodplain deposits of the upper Thames and its tributaries commonly comprise 0.5–1 m of clay alluvium overlying, with a sharp boundary, a variable thickness of sandy limestone gravel. Samples of wood (probably alder root) from the top of the gravel in a temporary exposure (SP 360084) in the floodplain of the river Windrush, south of Witney, Oxfordshire, gave a radiocarbon date of 2,660±85 yr BP (I-9337); these roots had been truncated at the gravel/clay interface. Here we consider the sequence of events and the possible causes of this change to clay deposition; we conclude that the latter resulted chiefly from a rapid increase in local forest clearance and in the amount of ploughed land, after 2,660 yr BP.

The Windrush is a tributary of the Thames; it drains the southern edge of the Cotswolds, Jurassic limestone hills north and west of Witney, and then flows southeastwards across the Oxford Clay country to the Thames at Newbridge (SP 403015).

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Its valley is characteristically narrow and entrenched within the Cotswolds but widens to about 1 km where it crosses the undulating clay land south of Witney.

The solid geology of the district is fairly simple. Thin Cornbrash limestone outcrops beneath Witney and to the north, with older limestones and clays of the Great Oolite series exposed locally in small folds and faults. These strata dip gently to the south-east and are overlain by Oxford Clay south of Witney. Drift deposits are extensive, especially the limestone gravels on river terraces.

About 1 m of Flandrian clay alluvium overlies the gravel in the Windrush floodplain adjacent to the river. Locally layers of peat, up to 0.3 m thick, occur at the junction of these two deposits, especially near the margins of the floodplain. Auger bores and occasional pits have proved this sequence of deposits throughout the Windrush floodplain from Witney to the Thames.

The site we sampled showed 1.06 m of clay alluvium over about 5 m of sandy limestone gravel, in turn probably over Oxford Clay (not seen); the gravel contained scattered organic matter. The root fragments were up to 0.04 m in diameter and seemed to be in position of growth in the gravel. They were truncated, only one fragment protruding some 0.1 m above the gravel surface.

These deposits suggest the following sequence of events. At first a high energy stream deposited the sandy gravel. Then followed peat accumulation, either locally in quiet backwaters or perhaps as a widespread deposit subsequently largely removed by erosion. Elsewhere in the Windrush floodplain we have seen large, unidentified logs in this peat, up to 0.1 m diameter. In either event the top of the gravel was eroded, at least locally, as in the section we studied the roots had been broken off and no tree stumps remained. The last event in this sequence was deposition of clay alluvium. A dark layer within it, about 0.5 m above the gravel and 0.07 m thick, represents an old topsoil—a level at which deposition temporarily slowed or ceased allowing organic matter to accumulate at the ground surface.

The radiocarbon date is significant for the age of the clay alluvium. Clearly the alder fragments post-date the gravel in which they grew, but are older than the period of erosion and the subsequent deposition of clay. The beginning of accumulation of clay alluvium at this site was, therefore, after 2,660 yr BP.

Corroborative evidence for changing conditions near or after this date is available at Farmoor, on the Thames 8 km east of this site. Here the Oxfordshire Archaeological Unit excavated an Iron Age site, dated to around 200 BC, which lies above the gravel surface but is buried by clay alluvium 0.3–1 m thick; mollusc, beetle and seed remains suggested that the site became very wet around 200 BC or possibly earlier[1].

Similar radiocarbon dates to ours have also been obtained from alluvial deposits, of a slightly different nature, at Ipsley, Worcestershire[2,8] at Pilgrim Lock, Warwickshire[3,8] and at Worcester[3,8], although here the alluvium seems to have accumulated at a faster rate than in the Windrush floodplain.

Although the sequence of events on the Windrush floodplain is reasonably clear, the cause of the change from the period of erosion which truncated the alder roots to the period of deposition of the clay is not. Four possible causes are considered below: lateral movement of the stream within its valley, a rise in sea level relative to the land, a change in climate, or a response to human activities.

Floodplain conditions in the Witney district were fairly uniform; the same sequence of deposits is recorded throughout much of the valley, and thus the changes must have been in response to factors of more than local significance. The available evidence[4] suggests that sea-level changes during this latter part of the Flandrian were very small, and unlikely to have significantly affected a river this far inland. The worsening of climate (higher rainfall) at about this time, the beginning of the sub-Atlantic period[5–7] could have been a contributing factor[5] but such changes alone are an unlikely cause. Climatic changes were possibly responsible for the cessation of erosion but there is no supporting evidence for the major change which would have been necessary to bring about this change in the nature of deposition, which seems to have lasted to the present, as the alluvium is still accumulating.

Although the deposits here are somewhat different our conclusions are similar to those of Shotton[8]. We suggest that the deposition of clay alluvium here began chiefly as a result of man's activity, probably a rapid increase in land clearance and cultivation (ploughing). This would have increased soil erosion, and have meant more rapid surface run-off and increased likelihood of short-term flooding in the river valley. Run-off would have been greatest over clay (impermeable) ground, so that much of the detritus carried to the river valley would be clay-sized (<2 μm) particles. This increase in land clearance and cultivation and the consequent deposition of the clay alluvium began after 2,660 yr BP.

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Oxygen 18 in estuaries

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A basic approach used in estuarine chemistry is to determine the experimental distribution of one given element with chlorinity and compare it with that predicted from the simple mixing of river water with seawater. This method provides useful results but several practical problems arise—including the definition of correct end-members, the role of input of tributaries or bank flow in the saline part of the estuary, and the occurrence of several water masses of different ages along the estuary. In addition, this method is not applicable in those areas of constant density which can be of prime importance in estuarine geochemistry and dynamic understanding. To characterise these various problems it is worthwhile to use a suitable freshwater tracer such as tritium[1], D/H or the $^{18}O/^{16}O$ isotopic ratio. The oxygen isotope ratio has been used successfully to study river water mixing[2] but has been little used in estuaries. The basic principle of this method is that the isotopic composition of river water might vary with time, and that different rivers might have different isotopic composition whereas ocean water is of fairly uniform composition. We present here a study of oxygen isotope composition in various estuaries, ranging (in Pritchard's classification[3]) from the saltwedge type such as Rhône (France) and Zaire (Congo), to a partially mixed type such as Gironde, Loire and Charente (France) and Godavari (India). These estuaries cover a wide variety of hydroclimatological conditions including the temperate and tropical regimes.

Samples were collected in Niskin bottles, or with a plastic bucket for surface samples. Aliquots of 25 ml were stored in tightly closed glass bottles for ^{18}O analysis and larger volumes were kept in polyethylene bottles for chlorinity measurements.

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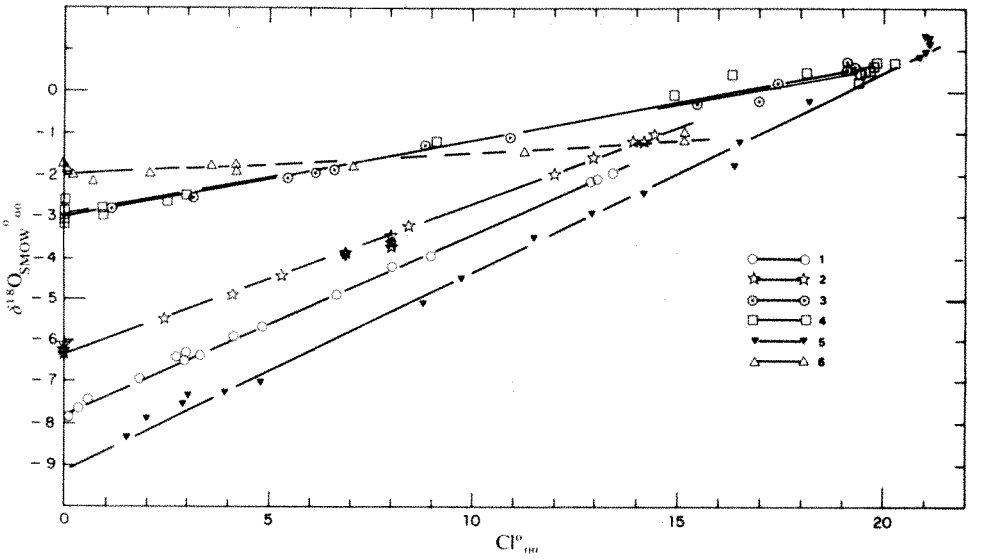


Fig. 1 ^{18}O content and chlorinity for: 1, Loire (30/04/1970); 2, Loire (23/11/1970); 3, Zaire (9–19/11/1976); 4, Zaire (7–13/05/1978); 5, Rhône (01–05/02/1978); 6, Godavari (13–17/12/1978).

The analytical procedure used in determining the $^{18}O/^{16}O$ isotopic ratio involves the equilibration of 3 ml of water with carbon dioxide gas at 25 °C and analysing a small aliquot of the gas in a mass spectrometer (VG 602 C)[4]. Oxygen isotopic ratios are expressed as per mil deviation ($\delta^{18}O$) from standard mean ocean water (SMOW), and the absolute accuracy of the method is ±0.05 δ‰.

Results of the analyses are presented in Figs 1–3 which systematically show the correlation of $\delta^{18}O$ with chlorinity. The estuarine classification based on the chlorinity structure[1] can be expanded by data on $\delta^{18}O$ distribution with chlorinity. It is possible to distinguish two categories of estuary:

(1) Estuaries with an isotopically homogeneous freshwater component (Fig. 1): in these estuaries the $\delta^{18}O$ experimental points fit fairly well the theoretical dilution curve. This most common type is observed for Zaire and Rhône estuaries which are typical salt-wedge estuaries according to chlorinity distribution. Results obtained in the Loire[5] and Godavari estuaries are given for comparison. These estuaries which are partially mixed and occasionally stratified according to chlorinity structure show a strict correlation between ^{18}O and chloride content. Thus in these four estuaries there is a perfect mixing between river and ocean water molecules. The slope of the regression curve for the Godavari estuary is much smaller, and extrapolation to the ocean water end-member—which it was not possible to sample at that time—gives values much lower than those shown on the Atlantic Ocean. However, a chlorinity end-member of 18.3‰ (ref 6) corresponds to a $\delta^{18}O$ of −1.0 using the Craig and Gordon equation[7] for the Pacific ($\delta^{18}O = 0.68S - 23.5$); the same using a similar equation developed by Duplessy[8] on the South Indian Ocean ($\delta^{18}O = 0.66S - 22.6$) would give −1.5‰. Therefore the Godavari regression line appears completely compatible with the local ocean setting.

(2) Estuaries with an isotopically heterogeneous freshwater component (Figs 2, 3): in this type of estuary $\delta^{18}O$ value of estuarine waters corresponds to the mixing of ocean waters with river water of various ages or origins: this has been observed in the Charente (1973) and Gironde (March 1978) where the rate of tidal mixing cannot smooth the small variations in river water isotope composition (Fig. 2). Moreover, in the Gironde (October 1978) and Charente (1974) (Fig. 3) well differentiated water masses can be identified. Considering first the Gironde (October 1978) waters with a chlorinity higher than 1‰ result from the mixing of ocean water with river water of $\delta^{18}O = -7$‰; below this chlorinity, the original river water has a $\delta^{18}O$ of −7.5‰. In the Charente (1974), the high chlorinity area corresponds to the mixing of ocean water with river water of $\delta^{18}O = -8.5$‰. For the domain with chlorinity below 7‰, the river end-member is characterised by $\delta^{18}O = -5.5$‰. A transitional zone exists between these two water masses. The water mass existing near the mouth which is located in the semi-enclosed basin of Marenne-Oleron results from the mixing of a 3 months old river water with $\delta^{18}O = -8.5$‰ with seawater.

Several factors may contribute towards the different isotope ratios encountered in river water. Some seasonal variations of the isotopic composition of precipitation reflect the parallel variation in the temperature of rain formation and sometimes in the relative humidity. The range of these long-term variations is

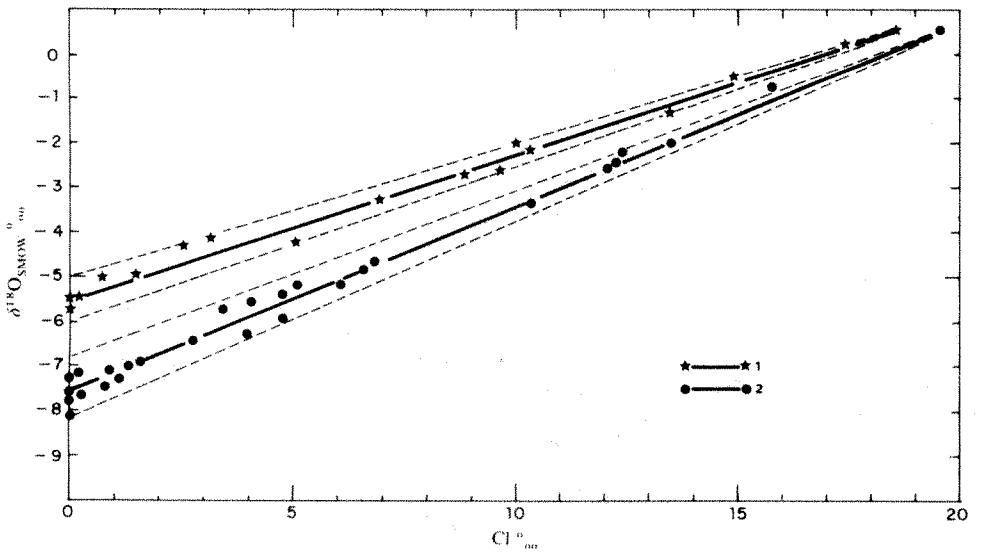


Fig. 2 ^{18}O content and chlorinity for: 1, Charente (22–26/10/1973); 2, Gironde (8–21/03/1978).

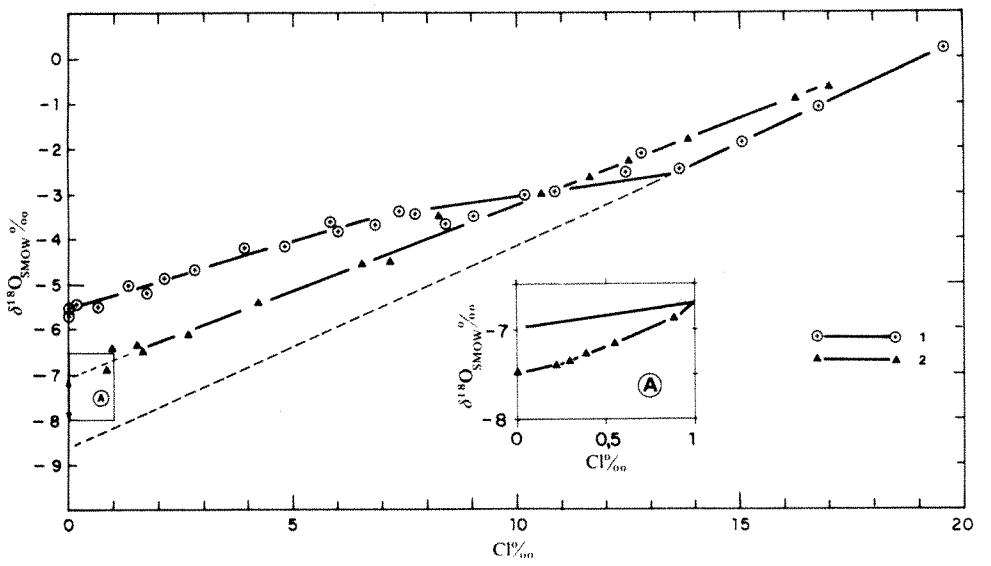


Fig. 3 ^{18}O content and chlorinity for: 1, Charente (22–24/07/1974); 2, Gironde (21–26/10/1978).

important, especially in continental areas[9]. Once they reach the drainage area, these variations can be smoothed by processes such as evaporation, mixing of rain water with ground and superficial waters, buffering through lacustrine retention, and possibly through exchange with host rocks and dissolved gases. However, these isotope ratios generally remain a good tracer for hydrological studies, summer waters being enriched in ^{18}O in comparison with winter waters. On a short-term basis, the isotopic composition of river water can also change as a result of a piston effect due to storm waters which flush out the water from soils and shallow aquifers: such variations reaching several δ‰ in ^{18}O have been observed in small alpine rivers[10].

As well as varying with time, the $\delta^{18}O$ isotopic ratio can also change, for a given watershed, with the distance from the ocean and with altitude. The effect of altitude which has long been recognised as an important factor in isotopic hydrology is basically related to changes in temperature. Together with the 'continental effect'[11] it provides an efficient tool to differentiate waters of different origins.

This first assessment of ^{18}O distribution in estuaries suggests a new approach in the study of water mixing, making it possible to differentiate between water masses along the estuary, including those in areas of constant chlorinity such as in the Gironde estuary (see above). It is then obvious that the comparison of the theoretical distribution curve with the actual distribution of one given element by using a single river water end-member can be misleading. This is shown by the dissolved Ca distribution in the Charente estuary in July 1974. At that time, a definite excess of Ca in the middle estuary was attributed to a dissolution process[12]. It is now clear from measurements of ^{18}O distribution that three water masses corresponding to river water of different chemical composition occurred along the estuary. Similarly, the PO_4^{3-} distribution, although biased by a severe pollution input, reflected the occurrence of the same water masses. In the Gironde estuary (March 1978) the river water samples which can be clearly defined through ^{18}O measurements, corresponds to a dissolved and particulate chemical composition very different from one sample to the other—for example, Zn, Cu and organic carbon, are respectively higher by factors of 1, 5 and 2 in the most downstream river sample (Cauwet *et al.* in preparation). More detailed analysis might provide a means of studying lateral mixing of different tributaries: in the Gironde estuary which receives two major rivers (Garonne and Dordogne rivers), the respective ^{18}O isotopic ratios during October 1978 survey were: ^{18}O-Garonne $= -7.75 \pm 0.27$‰ ($n = 17$) and ^{18}O-Dordogne $= -7.15 \pm 0.15$‰ ($n = 17$). In the case of Charente (1974) (Fig. 3) it was possible to estimate the estuarine water residence time.

The various freshwater components of estuaries might have different chemical compositions, so the classical comparison of actual chemical distribution of one given element with that predicted from the mixing of one single river water with seawater, can complicate geochemical interpretation. Systematic ^{18}O analyses should be of value in deciphering these problems. A limiting factor of this approach is the variation of the river isotopic signal, but in this study the variation was negligible in six of the estuaries described in this paper.

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Middle Pliocene climatic change in the western Mediterranean from faunal and oxygen isotopic trends

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The pelagic sedimentary sequences recovered by the Deep Sea Drilling Project (DSDP) from the Mediterranean are important because of their proximity to the classical shallow-water Neogene marine-type sections in Europe and because they may help correlations between the type sections and marine sequences outside the Mediterranean basin. We have studied the middle Pliocene (2.7–3.6 Myr ago) histories of surface-water temperature and oxygen isotopic composition at DSDP Site 132 in the Tyrrhenian Sea and here we compare these with another approach to estimating palaeotemperatures[1] based on the

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transfer function technique[2]. The record clearly shows a climatic cooling commencing between 3.2 and 3.0 Myr ago. An oxygen isotopic curve derived from the planktonic foraminiferan *Globigerinoides ruber* significantly correlates with a palaeotemperature record estimated from a transfer function palaeotemperature equation on planktonic foraminiferal data. Transfer functions may therefore be of value in interpreting palaeoclimatic history in sequences at least as old as the middle Pliocene.

Surface-water palaeotemperature estimates for Site 132 reveal relatively little temperature change before a cooling dated between about 3.1 and 3.2 Myr ago, followed by a slight warming over the Pliocene/Pleistocene boundary, and a cooling during the late Quaternary[1]. Previous studies of the Mediterranean late Neogene based on DSDP sites include semi-quantitative studies[3,4] and stable isotopic studies[5] of planktonic Foraminifera.

The transfer function technique has previously only been used to quantify Quaternary climatic variation[6]. The approach relates the distribution of microfossil assemblages in surface sediments of the ocean to the hydrographic conditions in overlying surface waters. Past temperatures at a given location may then be estimated based on changes in the microfossil assemblages with time. In extending this method to pre-Quaternary assemblages, the most fundamental assumption is that the fossil species lived in similar conditions to those of the present-day species. As a working hypothesis, we assume that species in evolving lineages and species which are similar in morphology to present-day species also have similar environmental preferences. Similar assumptions were made in a study of late Cenozoic palaeoceanography of the Panama Basin[7].

The middle Pliocene was chosen to isotopically test the reliability of pre-Quaternary palaeotemperature estimates using transfer functions for two reasons. First, middle Pliocene planktonic foraminiferal assemblages are significantly different from those of the Quaternary on which the transfer function is based. Thus, if our basic assumptions about morphologically similar species and evolving lineages of species are unjustified, the palaeotemperature estimates should become less accurate with increasing age. Second, the transfer function approach indicates a distinct cooling beginning about 3.2 Myr ago, a result which needed to be independently evaluated using stable isotopes.

Stable isotope stratigraphy using benthonic Foraminifera is based on correlating $\delta^{18}O$ variations which are inferred to largely reflect changes in continental ice volume[8]. During the Pliocene and early Quaternary the sill at the Gibraltar portal is thought to have been about 1 km deeper, based on ostracod assemblages[9], and a stable isotopic study of the earliest Pliocene at DSDP Site 132 (ref. 10). Therefore, the western Mediterranean basin probably exchanged deeper intermediate waters with the Atlantic Ocean during the middle Pliocene and the $\delta^{18}O$ of benthonic Foraminifera at DSDP132 should largely reflect ice volume variability.

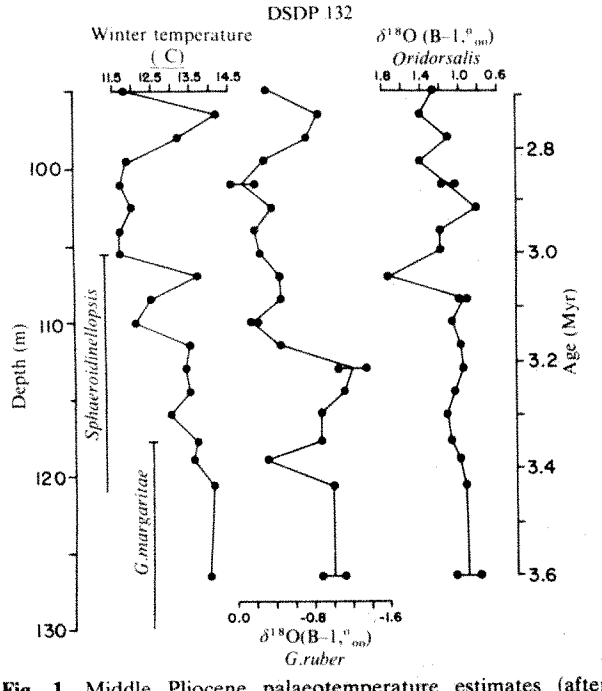


Fig. 1 Middle Pliocene palaeotemperature estimates (after Thunell[1]) and oxygen isotopic results at DSDP Site 132.

Table 1 Stable isotopic data (‰, B-1) for DSDP Site 132 samples

Sample (core-section, i.d. (cm))	Depth (m)	*Oridorsalis* $\delta^{18}O$	*Oridorsalis* $\delta^{13}C$	*G. ruber* $\delta^{18}O$	*G. ruber* $\delta^{13}C$
11-4 (49)	94.99	1.26	−2.50	−0.40	−1.04
11-5 (50)	96.50	1.40	−2.28	−0.94	−1.09
11-6 (50)	98.00	1.11	−2.29	−0.82	−0.74
12-1 (50)	99.50	1.41	−2.17	−0.38	−0.68
12-2 (50)	101.00	1.03	−1.75	−0.03	−0.55
		1.18	−1.95	−0.28	−0.39
12-3 (50)	102.50	0.82	−2.47	−0.45	−0.24
12-4 (50)	104.00	1.20	−2.05	−0.27	−0.37
12-5 (50)	105.50	1.19	−1.95	−0.33	−0.36
12-6 (50)	107.00	1.75	−2.41	−0.73	−0.52
13-1 (50)	108.50	0.92	−2.02	−0.55	−0.18
		1.00	−2.10		
13-2 (50)	110.00	1.08	—	−0.23	−0.35
				−0.30	−0.58
13-3 (50)	111.50	0.99	−2.16	−0.53	−0.35
13-4 (50)	113.00	0.96	−2.14	−1.43	−0.06
				−1.13	−0.22
13-5 (50)	114.50	1.05	−2.22	−1.20	−0.76
13-6 (50)	116.00	1.12	−2.17	−0.96	−0.24
14-1 (77)	117.77	1.09	−2.24	−0.96	−0.38
14-2 (41)	118.91	0.99	−2.11	−0.40	−0.55
14-3 (57)	120.57	0.93	−2.41	−1.09	−0.43
15-1 (53)	126.53	0.79	−1.94	−0.96	−0.02
		1.04	−2.01	−1.20	−0.01

Standard procedures were used in the stable isotopic analyses of this study[11]. Results are presented with respect to the powder standard B-1 because calibration of our working standard gas to PDB is incomplete at present. Friedman (personal communication to Shackleton[12]) has found the standard B-1 to be +0.29‰ with respect to PDB $\delta^{18}O$. Numerous analyses of B-1 give reproducibility (1 s.d.) of about ±0.06 for $\delta^{18}O$. Precision is slightly less for foraminiferal samples. *Globigerinoides ruber* was chosen (from the size fraction 175–350 μm) for analysis because of its persistant abundance throughout the Pliocene at DSDP Site 132 and because it is a modern surface-dwelling species, thus providing a surface-water temperature message. The benthonic foraminiferal genus *Oridorsalis* was chosen for analysis because of its relative abundance and because in another measured Cenozoic section this genus showed no fractionation of $\delta^{18}O$ relative to *Uvigerina*[11]. Likewise, Boersma and Shackleton[12] have reported no fractionation between *Uvigerina spinulosa* and *Oridorsalis umbonatus*. Unfortunately, *Oridorsalis* was rarely abundant enough to perform replicate analyses.

The chronological framework used in this study (Fig. 1) is based on previous calibration of planktonic foraminiferal events using a palaeomagnetic stratigraphy[13–15]. The most useful events are the extinctions of *Globorotalia margaritae* at approximately 3.3 Myr ago and *Sphaeroidinellopsis* at 3.0 Myr ago.

Stable isotopic results are presented in Table 1 and Fig. 1. Simple statistics comparing temperature estimates and isotopic results for the intervals before and after evidence of climatic change are presented in Table 2. The $\delta^{18}O$ of both planktonic and benthonic Foraminifera exhibits a sharp permanent increase in the middle Pliocene. Faunal evidence[1] and stable isotopic results on *G. ruber* suggest climatic deterioration began at about 3.2 Myr whereas it is dated at about 3.1 Myr based on

Table 2 Simple statistical comparison of oxygen isotopic data and temperature estimates before and after middle Pliocene cooling

	Post-cooling			Pre-cooling		
	mean	s.d.	*n*	mean	s.d.	*n*
δ^{18}O *G. ruber*	−0.49	0.24	12	−1.00	0.29	7
δ^{18}O *Oridorsalis*	1.25	0.26	9	1.01	0.07	10
Winter *T* (°C)*	12.4	0.89	11	13.6	0.35	8

*After Thunell[1].

Oridorsalis isotopic results (Fig. 1). Thus, evidence of pronounced cooling and ice volume increase occurs within an interval of about 0.1 Myr in the western basin of the Mediterranean.

The benthonic oxygen isotopic record between 3.1 and 3.6 Myr is highly stable and similar to other benthonic δ^{18}O records of the same age elsewhere. Thus, there is strong evidence that the Pliocene between 3.1 and 3.6 Myr ago was a time of relatively constant ice volume and bottom-water temperature[11,16,17]. The fact that these Mediterranean results are similar to those outside the Mediterranean basin supports Benson's[9] concept that deep, cold (psychrospheric) Pliocene ostracod assemblages in the Mediterranean indicate a deeper-water connection across the Gibraltar sill. At about 3.1 Myr the δ^{18}O of benthonic Foraminifera increases by about 0.8‰ and on average by 0.24‰ (significant at the 95% level) and becomes much more variable (Fig. 1, Table 2). The change at 3.1 Myr is interpreted as reflecting the first major increase in permanent Northern Hemisphere ice volume, supporting previous studies outside the Mediterranean[11,18–22]. The detail of the DSDP Site 132 isotopic record, especially the sudden inferred ice growth between about 3.0 and 3.1 Myr is very similar to the records obtained at DSDP Sites 310 (ref. 11) and 397 (ref. 22). The increased variation in the benthonic δ^{18}O signal reflects an increase in climatic variation after 3.1 Myr as polar ice caps changed in volume. Evidence from glacial tills overlain by lavas[18,19] and from deep-sea cores[20,21] suggests that this first ice growth occurred between about 3.0 and 3.2 Myr ago, and is supported by our results.

The permanent isotopic enrichment recorded by *G. ruber* occurs at 3.2 Myr, predating that exhibited by *Oridorsalis* by about 0.1 Myr (Fig. 1). After about 3.2 Myr the δ^{18}O of *G. ruber* is enriched by an average of 0.51‰ (Table 2) which we interpret to reflect a decrease in surface-water temperature in addition to the compositional effect recorded by *Oridorsalis*. The oxygen isotopic record of *G. ruber* correlates well ($r = 0.79$, significant at the 99% level) with the winter palaeotemperatures calculated by Thunell[1], using the transfer function approach. A comparison of these curves (Fig. 1) shows that the sense of climatic change is the same but the amplitude is often different. Such differences in amplitude may result from the effects of evaporation and precipitation on δ^{18}O. If we assume that the average isotopic enrichment of *Oridorsalis* after the onset of climatic cooling (0.24‰) represents only an ice volume change, and that the average enrichment of *G. ruber* (0.51‰) reflects an additional temperature effect, then the difference between the two (0.27‰) should be an estimate of the average surface-water oxygen isotopic change resulting from decreased temperatures after about 3.1 Myr ago. An isotopic change of about 0.27‰ represents about a 1 °C cooling, in good agreement with the average cooling estimated by faunal means (1.2 °C; Table 2). Such agreement must be demonstrated for larger coolings and over longer time intervals. In general we believe that the transfer function approach is valuable for determining palaeotemperatures in Pliocene sequences, when the evolution of species lineages is taken into account.

The lag of about 0.1 Myr between the enrichment in the benthonic δ^{18}O record and the cooling indicated in both the palaeotemperature and planktonic δ^{18}O records indicates that a cooling of surface waters in the Mediterranean, and possibly increased evaporation relative to precipitation, predated the build-up of permanent Northern Hemisphere ice sheets. The development of climatic cooling immediately before the initiation of Northern Hemisphere ice growth is also manifested as an intensification of abyssal circulation at approximately 3.3 Myr[23].

A comparison of the benthonic δ^{18}O record and the surface-water palaeotemperatures reveals that the initial growth of permanent ice occurred during a time of warm surface waters. A similar relationship has recently been observed by Ruddiman and McIntyre[24] in which they found periods of most rapid ice growth during the late Quaternary in the North Atlantic were associated with warm surface conditions. We speculate that warm surface waters may have provided a necessary source of moisture for the first permanent accumulations of Northern Hemisphere ice sheets.

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Nest of juveniles provides evidence of family structure among dinosaurs

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One of the greatest mysteries associated with dinosaurs is the rarity of juveniles. With the notable exception of the Djadochta and Iren Dabasu Formations of Mongolia and the Two Medicine Formation of Montana, skeletal elements of very young dinosaurs are exceedingly uncommon[1–3], leaving a gap in the understanding of dinosaur social structures[1,3,4]. The most popular explanation, based chiefly on negative evidence, is that the eggs and young were 'kept' in the uplands where they were rarely covered by sediments and as a result were destroyed by erosion[2]. The discovery of fifteen 1-m long hadrosaurian ('duck-billed') skeletons together in a nest-like structure offers the first tangible evidence of the social behaviour of at least one group of dinosaurs. They were discovered by Marion Brandvold in terrestrial sediments of the Two Medicine Formation (Upper Cretaceous) near Choteau, Teton County, Montana.

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The skeletons of 11 little hadrosaurs were found jumbled together in a fine-grained green mudstone that infilled a bowl-shaped depression in a fine-grained brown mudstone (Fig. 1). The concave structure, oval in outline, was about 2 m in diameter at its weathered surface and extended about 0.75 m deep near its centre. Portions of four other individuals were scattered around the weathered surface, no more than 2 m away. Measurements of the skeletal elements, particularly the femora, indicate that all individuals were essentially the same size. The uncrushed femora vary between 120 and 130 mm long. Within the surrounding green mudstone were several minute fragments of eggshell and three terrestrial gastropods (family Polygyraeidae). Although it may have been an artefact created by differential weathering of the sediments, the concave structure was situated on the apex of a mound-shaped 'hill' approximately 3 m in diameter and about 1.5 m above the surrounding topography.

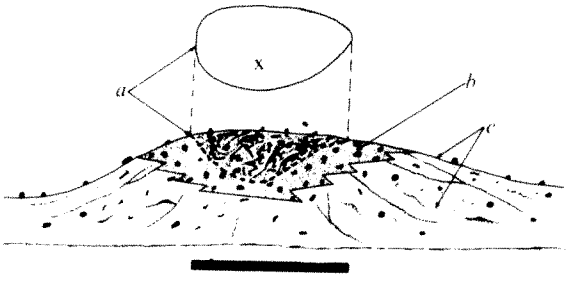


Fig. 1 Reconstruction of nest. *a*, Outline of green mudstone which contained the baby dinosaurs, eggshell and gastropods; *b*, brown mudstone that surrounded the green mudstone; *c*, weathered surface. (Scale bar, 2 m.)

The presence of several small skeletons, apparently all the same species and size, together with eggshell in an oval, concave structure, strongly suggests that the structure was a nest. The skeletons (Fig. 2) have several characteristics indicative of very young individuals. The sacral vertebrae are not fused; the neural arches are not fused to the vertebral centra; the proximal and distal ends of the limb bones are not well-formed, and the absence of the basicranial bones suggests that they were not ossified[5,6]. Nevertheless, there is evidence that the young were capable of activity outside the nest. With the exception of the basicranial elements, all the skeletal elements present in an adult hadrosaur were already ossified, including the carpals and tarsals and the ossified tendons that run along the sides of the neural spines of the posterior dorsal, sacral and caudal vertebrae. The feet are somewhat large for the size of the animal, but the limb proportions are very similar to those of adult hadrosaurs, suggesting that very little ontogenetic change occurred in the postcranium during growth. Wear on the occlusal surfaces of the dental batteries indicates that the young had been feeding for some time. Some of the individual teeth are worn more than three-quarters their length. Another group of juvenile hadrosaurs found in the same general area, although several hundred metres from the nest, indicate that these nestlings are at least twice the size of hatchlings. Among the elements (PU 22432) are three femora averaging 69 mm in length, indicating that the animals were about 450 mm long. A considerable number of large eggshell fragments was found with the specimens.

The fact that 15 baby hadrosaurs had been feeding, and had stayed together for a period of time, indicates that some form of extended parental care was administered for, if the young were confined to the nest, food must have been brought to them. If the young had ventured out to forage for food, it is unlikely that they would have returned to the nest together without parental supervision.

Extended parental care is suggested by several other age-cohort associations of young dinosaurs in the same formation and area. Gilmore[7] wrote that a mounted skeleton of the horned dinosaur *Brachyceratops montanensis* (USNM 7951) was made up of the bones of at least five individuals, all from the same deposit, all belonging to one species and all practically the same size. *Brachyceratops* is considered to be a juvenile of *Monoclonius*[8] which averages about 4.2 m long, or slightly more than twice the length of the juveniles described by Gilmore. Of a second occurrence, Gilmore[9] described a stratum full of scattered bones representing many individuals of a small hadrosaurian dinosaur. Measurements of the limb elements indicate that the individuals (USNM 16600) were about half the length of an average adult hadrosaur. The average length of the femora is 500 mm. Another group of hadrosaurs (USNM field no. Qu-3-35) collected by Gilmore consists of portions of at least three individuals each slightly less than half the length of an adult. The American Museum of Natural History has three individual hadrosaurs (AMNH 5358) which each have a femur length of about 450 mm.

All these groups of dinosaurs were collected from the Two Medicine Formation, no more than 95 km north of the nest described here. Of the more than 200 specimens from this formation now in the US National, American and Princeton Museums, more than 80% seem to pertain to individuals one twentieth to half the linear dimension of their adult counterparts. Eggshell fragments are the most common fossils in the formation and whole or partial eggs are not uncommon. The abundance of eggshell and juvenile hadrosaurs and ceratopsians in these sediments, as opposed to the scarcity of them in the adjacent Judith River (Oldman) Formation of Alberta[2,10,11] and Montana (our observations), suggests that the depositional area of the Two Medicine Formation was a nesting ground for at least these two groups of ornithischian dinosaurs.

If the juvenile dinosaurs collected by the US National and American Museums from the Two Medicine Formation represent nestlings similar to those described here, parental care may have been extended until the young were about half the linear dimension of their adult counterparts, which suggests that the parent or parents spent considerable time with the young, or that the young grew very rapidly. As with birds, a rapidly growing dinosaur would have required a very high metabolic rate (endothermy), a considerable supply of food and a very efficient means to process the food. Efficient mastication seems to have been highly specialised among the hadrosaurs and ceratopsians, both of which possessed teeth able to pulverise and grind considerable quantities of food (? plant material). This may indicate that the other ornithischian dinosaurs, such as ankylosaurs and other ornithopods which do not have complicated dental systems, either grew more slowly or had other means for efficient food processing.

Because of the immaturity of the juvenile dinosaurs and the ontogenetic changes which probably occurred in the skulls during growth, a specific identification of the 15 individuals from the nest may not be possible until more specimens of older individuals are found. Characteristics of the skulls, however, suggest that they are generically similar to a large skull (Fig. 2) found within 100 m of the nest. The large skull represents a new genus and species and, although it has some rather peculiar characteristics, is placed in the subfamily Hadrosaurinae.

Maiasaura (new genus)

Holotype: PU 22405, skull with partial right dentary and predentary.

Referred specimens: PU 22400, skeletons of 15 individuals.

Horizon: Two Medicine Formation, Upper Cretaceous (Campanian).

Locality: 12 miles west of Choteau, Teton County, Montana, on the James and John Peebles ranch.

Collectors: J. R. Horner and R. Makela, 1978, for the Museum of Natural History, Princeton University.

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Etymology: Maia from the Greek which means good mother, saura which means reptile (feminine).
Diagnosis: Wide elongate facial region. Short naris located on anterior end of snout with an extensive lateral facial region between the posterior end of the naris and the anterior end of the orbit. Short wide premaxillary bill with deflected border similar to that of lambeosaurines. Long shallow maxilla with an anterior maxillary process and anterior maxillary notch as present in hadrosaurines. Small, solid incipient crest above and between orbits, formed by the nasal and frontal bones. Nasals concave anterior to incipient crest. Quadratojugal process of the jugal long, thin and angling steeply upward to meet quadratojugal. Dentary teeth with long and narrow enamel crown. Predentary wide and very shallow with round, evenly spaced, denticulate processes around its anterior edge.

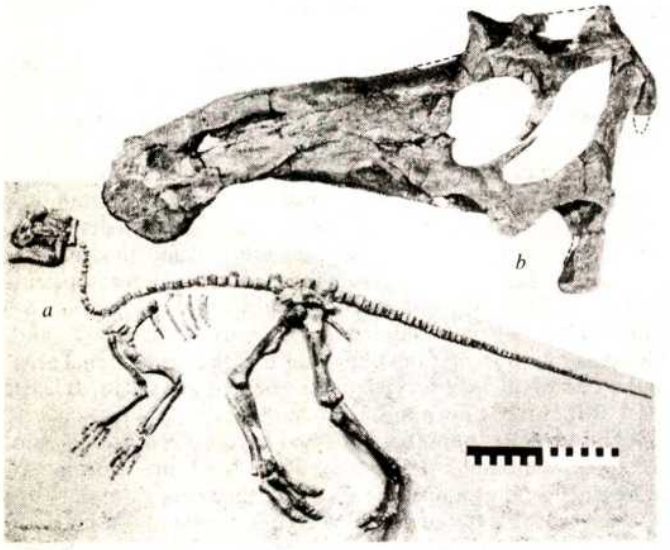


Fig. 2 *Maiasaura peeblesorum*. *a*, Incomplete reconstructed composite skeleton of individual from nest (PU 22400); *b* holotype skull (PU 22405). (Scale bar, 20 cm.)

Maiasaura peeblesorum (new species)
Specific characters same as generic characters.
Etymology: Specific name to honour the James and John Peebles families, owners of the land where the specimens were collected.
Discussion: The skulls of the referred juvenile specimens have short, wide deflected premaxillary bills, long shallow maxillae with anterior maxillary processes and notches and long, thin, steeply angling quadratojugal processes of the jugals. In all these characters they agree with the adult specimen. The juvenile skull elements are however disarticulated and in many instances crushed so that a positive identification cannot be made.

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The Cossack skull and a dihybrid origin of the Australian Aborigines

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Recent surveys of Australian prehistory[1–4] reveal the interest and debate surrounding the evolutionary implications of the observed range in cranial morphology of modern Australian Aborigines and their predecessors. In spite of an earlier model postulating a trihybrid origin[5,6], a theory favouring a homogeneous colonising stock had gained some acceptance[7,8]. Discoveries[9,10] of the past decade raised doubts about the validity of the homogeneity concept and suggested an alternative two-population—gracile and robust—model[11–13]. However, as all the material described was derived from a restricted area of South-East Australia, the possibility existed that the apparent dichotomy might reflect isolate variation or local adaptation[14]. We discuss here recently described human skeletal material from Cossack, Western Australia[15] which has a demonstrable affinity with the postulated South-East Australian robust group, making local adaptation/population isolate explanations unlikely. The Cossack skull supports arguments favouring an early dihybrid origin with substantial change in Australian Aboriginal cranial morphology since the Pleistocene.

Discovered 3 km ESE of Cossack, Western Australia (Fig. 1), the material comprises most of a cranium and mandible together with fragments of the shafts of several limb bones. The skull was lying at the base of an eroding coastal dune and fragments of the long bones were scattered over the lower face of the dune. Direct dating of this individual is not feasible at present. However, the region's coastal features indicate that rising post-Pleistocene sea levels first reached the contemporary coastline around 6,500 yr BP[16], thus apparently restricting the material's age to a period after that event.

Most areas of the skull are represented except for the base, the central portion of the face, and the left temporal, zygomatic and orbital regions; almost all the teeth are present. After cleaning and restoration, it was apparent that inward compression of the whole left side of the skull had occurred after death. However, the right side is relatively complete and undistorted, and some metrical observations are possible. On morphological criteria[17], the individual is clearly male and was about 40 yr old at death.

The Cossack skull (Western Australian Museum no. A22684) includes a massive dolichocephalic calvaria with a large and slightly prognathous face (Fig. 2). The most striking feature is the markedly backward-sloping forehead. The supra-orbital margins are well developed and slope back strongly from the glabella to the large zygomatic trigones. Other features include some occipital bunning, an occipital torus, a clearly marked temporal line, a large supra-mastoid crest and a prominent malar tuberosity. The mandible is correspondingly large and robustly built, and the dentition shows heavy wear. The right 1^1 is missing and the alveolus completely resorbed, suggesting the possibility of tooth evulsion for cultural purposes as practised by recent Australian Aborigines.

Metrical comparison of the Cossack skull with a series of recent male Western Australian Aborigines[18] reveals several

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significant differences (Table 1). Cranial length is more than 5 standard deviations greater than the series mean. Although estimated breadth is slightly greater than the maximum Western Australian value, the cranial index of Cossack is comparable to the series lowest figure. Parietal size (bregma-lambda chord) is close to 3-standard deviations greater than the Western Australian mean. The Cossack skull's frontal curvature index is more than 5 standard deviations below the series minimum value. Cranial bone thicknesses are considerably greater than those of recent Australian Aborigines[15]. The most notable metrical mandibular comparison is of the corpus projective length, which yields a value more than 3 standard deviations greater then the series mean.

The features in which the Cossack skull differs from recent Western Australians are precisely those in which it resembles certain prehistoric South-East Australians (Table 1). Low frontal index and great length (the Cossack skull has the highest Australian values yet recorded) as well as general robusticity, highlighted by cranial vault bone thickness, are characteristic of the postulated robust group, including Talgai, Cohuna, Mossgiel and Kow Swamp. Comparison of lateral cranial contours[15] reinforces the referral of the Cossack skull to this group.

The demonstrable affinity of the Cossack skull to the robust South-East Australian group indicates that this morphology was not a regional variant but continental in distribution. This newly established range strengthens the evidence for a two-population occupation of Australia.

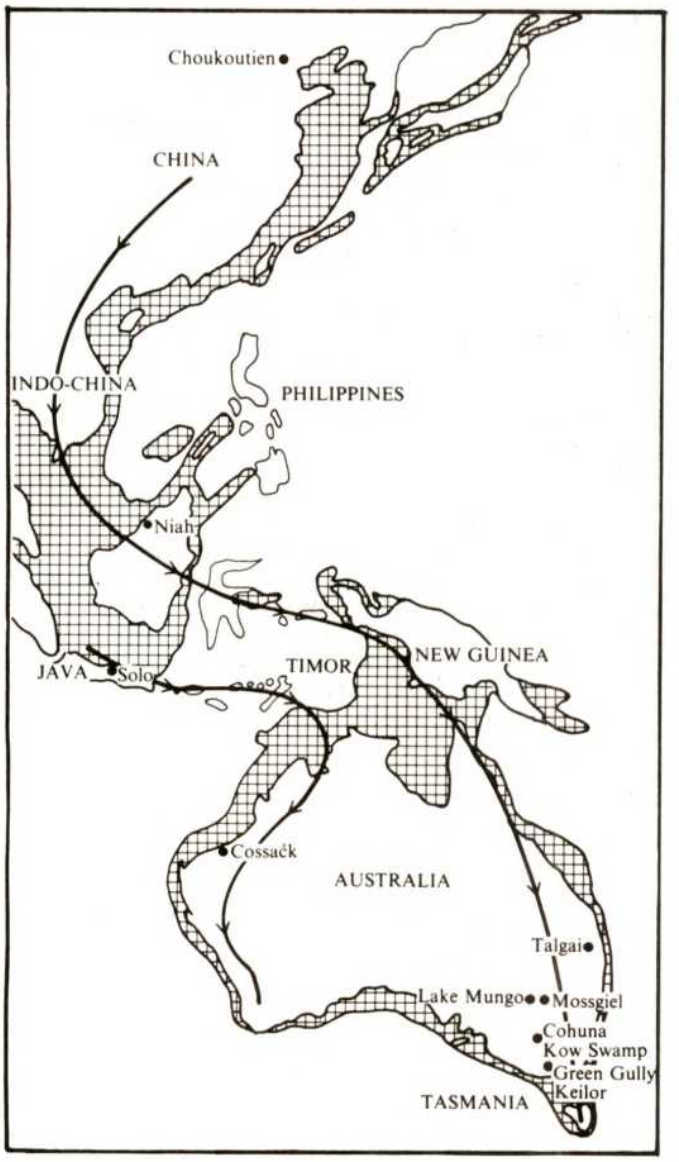


Fig. 1 Present and late Pleistocene (hatched) land areas. Fossil hominid sites and possible migration routes to Australia.



Fig. 2 Lateral view of the Cossack skull.

It is now generally accepted that human colonisation of the Australian continent first occurred at least 40,000 yr ago and probably earlier[3]. Fossil evidence in Australia of a more robust population extends from about 13,000 yr BP (Kow Swamp) to 6,000 yr BP (Mossgiel) and now possibly to even more recent times (Cossack). For the more gracile group the time span now ranges from 26,000 yr BP (Lake Mungo) to 13,000 yr BP (Keilor) to 6,000 yr BP (Green Gully)[8,11,13].

The two Asian stocks from which these populations could have been derived are: (1) a South-East Asian population, perhaps from Java and adjacent areas, and (2) a more northern population, possibly from southern China. These would be early *Homo sapiens* populations perhaps differing morphologically from each other as do the earlier Javanese and Chinese *Homo erectus* people. The more robust South-East Asian migrants, possibly morphologically related to the Solo (Ngandong) people[19], could have taken a southerly route from Java by way of Timor into northwestern Australia and then down the west coast (Fig. 1). The more gracile population from southern China may have followed a more northerly course, perhaps passing through Indo-China, Borneo and New Guinea before arriving at the northeastern part of Australia. They would then have passed down the east coast and perhaps finally across the land bridge to Tasmania (Fig. 1). The 40,000-yr-old Niah cranium[20] from Borneo and the apparently more gracile appearance of the recent Tasmanians[11] may be the best evidence of this migration. These two early colonising Australian populations may have used different adaptive strategies. Their subsequent fusion might have been triggered initially by the climatic and faunal changes of the late Pleistocene but was only completed in recent times[22].

Table 1 Selected metric comparisons

Measurement	Cossack[15]	Male West. Aust.[18] (range)	Kow Swamp[21] (range)
Max. cranial length	220	169–202	190–214
Max. cranial breadth	(145)	117–143	128–150
Bregma-lambda chord	131	104–128	115–126
Cranial index	65.9	64.64–76.33	(66.67)–72.39
Frontal curvature index	12.6	18.58–25.66	12.71–16.12
Mandibular corpus projective length	98	71–95	

Measurements are given in mm.

Rapid advances have been made in the study of the prehistory of the Australian Aborigines over the past 10 yr. Syntheses of the new material and concepts[1-4,11-13] are now bringing closer an understanding of the people who in the latter part of the Pleistocene colonised one of the last two major unoccupied land masses.

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Thermal buffering in Afro-alpine plants due to nucleating agent-induced water freezing

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Plants in the alpine zone of Mount Kenya are exposed throughout the year to environmental temperatures varying from about −10 to +10 °C (ref. 1). In contrast to plants in Arctic and temperate regions, which are in a state of suspended growth during the cold season, these plants have to combine growth with tolerance to low environmental temperatures. It has been suggested that flowers of the Afro-alpine plant *Lobelia telekii* gain thermal protection from the long, thin bracts which hang down at the outside of the inflorescence, and which seem to lower the radiative heat loss of the plants at night[1]. We report here that vital tissues of *L. telekii* are also protected by the heat of fusion of water, which is present in the central part of the inflorescence, and that freezing of this water is ensured by the presence of nucleating agents, which induce freezing at a few degrees below zero.

The inflorescence of *L. telekii*, which may reach a height of 2 m, is cylindrical and hollow, with an inner diameter of 5–8 cm (Fig. 1*a*). The central cavity contains a slightly viscous fluid, which is apparently produced by the plant. The fluid level within the plant reaches more than 0.5 m above the ground, and several litres may be drained from each inflorescence. The temperature of the air and in the central part of a flower and that of the central fluid in the inflorescence of a *L. telekii* plant were measured during the day by means of thermocouples mounted in fixed locations. Temperatures recorded are shown in Fig. 2. After sunrise the temperature in the flower rose to several degrees above that of the air, indicating that the bracts are also important for absorbing radiant heat from the Sun[2]. The absorbed heat also gradually leads to an increase in the temperature of the central fluid. As the air temperature dropped in the afternoon, the temperature in the central fluid lagged behind. In contrast to the temperature in the air, the temperature in the flower and in the central fluid did not drop below zero during the night; the temperature in these parts of the plants levelled out at zero and stayed there until sunrise.

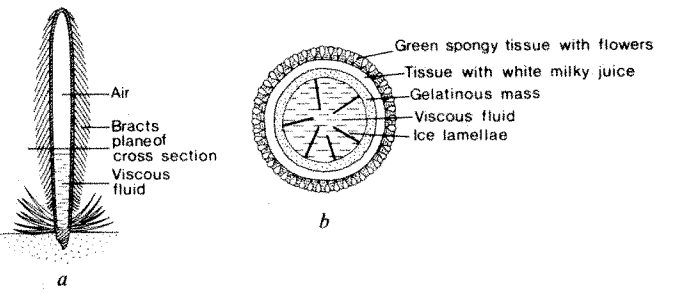


Fig. 1 Longitudinal section (*a*) and cross section (*b*) of *Lobelia telekii*.

The time lag between the drop in air temperature and the fall in temperatures measured in the plant is mainly due to the high heat capacity of the central fluid. This time lag, as well as the stabilisation of the temperature of the plant at zero, imply that the flowers are thereby protected from exposure to the low and possibly injurious air temperature.

When the inflorescence of a *L. telekii* plant was cut just above the surface level of the central fluid at sunrise, the central fluid was found to contain ice, in the form of vertical thin lamellae, which protruded radially into the central fluid from the wall of the inflorescence, as indicated in Fig. 1*b*. The amount of ice was estimated at ~2% of the fluid volume. Liberation of the heat of fusion of the frozen water leads to a stabilisation of the temperature at the melting point of the unfrozen fraction of the central fluid. Thus the formation of ice in the central fluid and the concomitant liberation of the heat of fusion form the physical basis for the stabilisation of fluid and flower temperature at about 0 °C during the night. The temperature of the partially frozen central fluid was 0.1 °C, indicating that in spite of its viscous character, the central fluid has a very low osmotic activity. This will ensure maximal thermal protection of the plant by leading to a stabilisation of the temperature of the frozen central fluid at the highest possible subzero temperature.

The freezing of the central fluid will in turn also have a thermally buffering effect on the air-filled compartment above

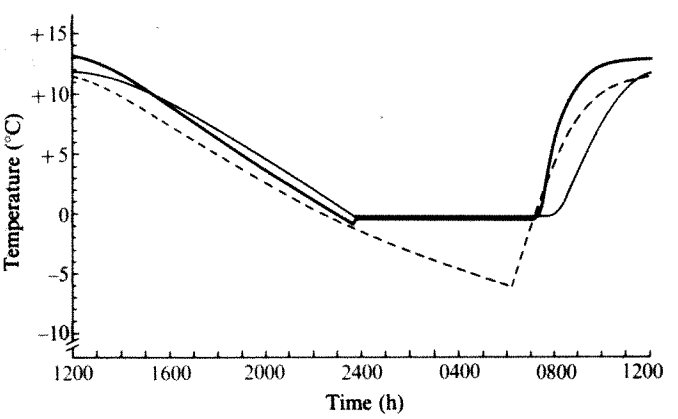


Fig. 2 Temperature variations of the air (dashed line), the central part of a flower (heavy line) and the central fluid in the inflorescence (thin line) of a *Lobelia telekii* plant.

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the central fluid (Fig. 1*a*). As the air is trapped inside the inflorescence and cannot escape, it will circulate inside the upper part of the inflorescence and cause internal heating at the top of the plant. Thus, the heat of fusion liberated in the central fluid will contribute not only to the thermal protection of those parts of the plant which surround the central fluid, but also to the whole inflorescence.

Based on these observations, it seems to be of importance to the survival of the plant to induce freezing in the central fluid at the highest possible subzero temperature. Freezing at high temperatures and prevention of supercooling are known to be affected by the presence of so called nucleating agents. A search for such agents in the internal fluids from the plant was initiated, using a method described by Zachariassen and Hammel[3]. Samples of central fluid (0.25 μl) were transferred to 5-μl samples of 0.9% NaCl solution, and the supercooling point of the mixed samples was measured. The supercooling point of four parallel samples was -4.6 ± 1.3 °C (±s.d.), whereas samples of undiluted NaCl solution supercooled to −15 to −20 °C. This revealed that the central fluid of the inflorescence of *L. telekii* plants must contain nucleating agents, which will induce freezing at as high temperatures as possible.

The nucleating effect of the central fluid was still intact following heating of the fluid to +100 °C for 5 min. Investigations of the chemical composition of the central fluid revealed that the fluid contains about 15 mg ml^{-1} of carbohydrates, as determined by the phenol sulphuric acid colour method[4], using glucose as standard. The content of protein was found to be about 0.15 mg ml^{-1} by the FC analysis[5], using bovine serum albumin as a standard. This low protein value agreed with analysis for nitrogen on a Carlo Erba elemental analyser Model 1104. A fraction of the organic material was non-dialysable, indicating that the central fluid contains a high molecular weight polysaccharide as one of the components. The high heat stability of the nucleating agents, combined with the quantitative domination of carbohydrates in the fluid, might indicate that the nucleating agents are carbohydrates.

After a standard hydrolysis (1 M H_2SO_4, + 100 °C, 5 h), the monomeric sugars in a yield of about 30% were separated as glycitol hexoacetates by gas chromatography. By co-chromatography, the main glycitols were shown to be iditol, glucitol and mannitol. The carbohydrates gave a strong positive reaction in a test for keto sugars[6]. Work to identify the sugars and to elucidate the constitution of the polysaccharides is being carried out.

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Melatonin, the pineal gland and human puberty

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Animal experiments have suggested that the pineal gland produces an anti-gonadotropic hormone. The hamster, for example, undergoes reproductive collapse when kept in short-day periods, an effect which is abolished by pinealectomy[1]. Although there is little direct evidence about the endocrine role of the pineal gland in man, it has been noted that tumours of the pineal gland in young boys are associated with precocious puberty and the human pineal gland has been suggested to produce a substance that holds sexual maturation in check[2]. This observation has been extended by Kitay[3], who has shown that destructive tumours are associated with precocious puberty whereas hyperactive tumours are associated with delayed puberty. However, no studies have described any change of pineal function with normal puberty. Because two pineal indoles, melatonin[4] and methoxytryptophol[5], have been shown to be antigonadotropic when administered to animals[6–10], we have now measured them in schoolchildren. Our findings show that in young boys there is an abrupt fall in the concentration of melatonin with advancing development suggesting that it may play an important physiological role in the control of human puberty.

We studied 51 healthy boys and girls between the ages of 11½ and 14 yr; all attended a normal secondary school in Somerset, UK and blood was taken between 1100 and 1300 hr. At the time of sampling each child was assigned to one of five maturity stages[11] according to genitalia or breast development. Some of the children were studied on up to four occasions such that an overall total of 101 blood samples was taken. In addition to pineal hormones, serum luteinising hormone (LH), follicle stimulating hormone (FSH), testosterone and oestradiol were measured by radioimmunoassay. Melatonin and methoxytryptophol were assayed by gas chromatography–mass spectrometry (GCMS)[12,13] (Fig. 1). The sensitivity of the assay was assured by single ion monitoring at *m/e* 232 because this was the most abundant fragment ion in the mass spectra of melatonin and methoxytryptophol. It was also a fragment characteristic of the methoxyindole nucleus and therefore contributed to the specificity of the assay. Specificity was further enhanced by increasing the resolution to 2,000 so that the more precise mass 232.12 was monitored. Selected samples from the schoolchildren which gave high values for melatonin using the routine assay (monitored at *m/e* 232) were validated by being re-assayed at *m/e* 245 and *m/e* 304 and results were consistent with those previously obtained. The ratio of *m/e* 232 to *m/e* 245 was 1.23 ± 0.16 s.d. (synthetic melatonin 1.21 ± 0.04).

The concentration of methoxytryptophol ranged from <4 to 92 pg ml^{-1} in the boys and from <2 to 400 pg ml^{-1} in the girls and did not show a sex distribution or any change with development. The concentration of melatonin in the schoolgirls ranged from <5 to 280 pg ml^{-1} and did not change with development. In contrast, the concentration of melatonin in the boys which ranged from <10 to 2,300 pg ml^{-1} showed a marked change during adolescent development (Fig. 2). The means and 95% confidence limits were calculated from log transformed data by mixed longitudinal methods described by Tanner[14]. At genitalia stage 1 the mean value of melatonin was 218 pg ml^{-1} (95% confidence limits 509–93, $n = 13$). At stages 2, 3 and 4 the mean values of melatonin were 30 pg ml^{-1} (128–18, $n = 28$), 17 pg ml^{-1} (24–13, $n = 15$) and 18 pg ml^{-1} (32–11, $n = 11$). At stage 5 there were only two samples from the school population and to these were added the daytime values from normal adults, making a mean of 16 pg ml^{-1} (11–21, $n = 18$). The fall in concentration of melatonin from stage 1 to stage 2 was highly significant ($P < 0.001$), but no significant changes occurred thereafter.

When the concentration of melatonin was tabulated against the concentration of LH assayed in the same samples (Fig. 3), the mean value of melatonin decreased from 287 pg ml^{-1} to 24 pg ml^{-1} when the concentration of LH increased from

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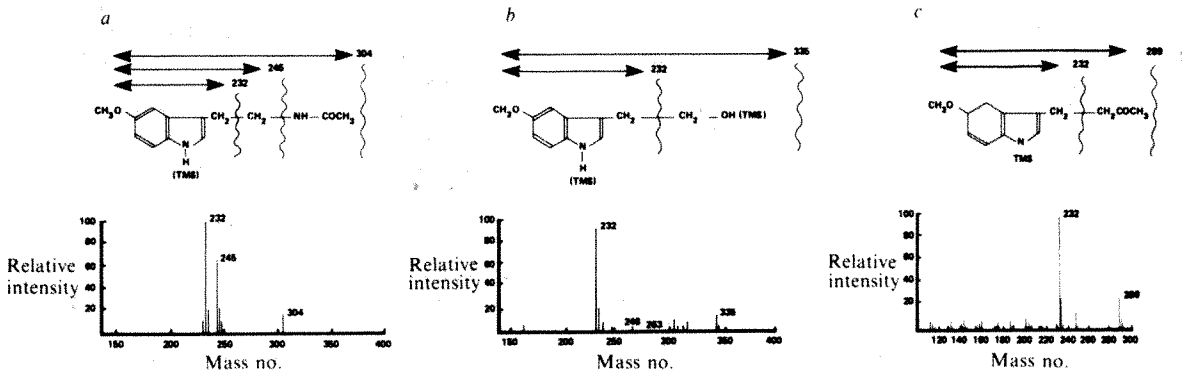


Fig. 1 Molecular structures and partial normalised mass spectra of TMS derivatised melatonin (*a*), methoxytryptophol (*b*) and the internal standard [3-(butan-3'-one)5-methoxyindole] (*c*) are illustrated. Internal standard (50 ng) was added to 1 ml of plasma or serum sample and vortex mixed with 3 ml of chloroform. The organic phase was separated and evaporated to dryness. Before assay the dried extract was reconstituted in 10 μl of silylation reagent. Portions of 5 μl were injected on to GCMS. Methoxytryptophol entered the ion source at 2 min 15 s, the internal standard at 2 min 40 s and melatonin at 4 min 40 s. The magnet and electrostatic analyser were tuned to allow only the passage of ions of mass 232 (strictly m/e 232). Each sample was quantified by determining the ratio of the peak height of endogenous melatonin or methoxytryptophol to the peak height of the internal standard and calculating the concentration from a standard curve.

undetectable values to values measurable but still below 1 IU l^{-1}. This fall in concentration of melatonin was highly significant ($P \ll 0.001$). The analysis of the fall in concentration of melatonin relative to increasing levels of FSH or testosterone showed the same pattern but the association was less striking.

We have reported a limited study and further work is required, especially in relating these findings to circadian changes. However, it is already clear that, using the same GCMS assay for melatonin as reported here, the levels of melatonin found in most samples from the prepubertal schoolboys exceed anything measured in the day or night from normal adult volunteers who have been monitored in a sleep laboratory (P. E. Mullen, unpublished observations). Also, studies on the circadian values of melatonin in pubertal boys show values which are no different from those of the mature adult[15]. However, ethical considerations have prevented investigation of prepubertal boys in the sleep laboratory where it would have been valuable to relate the circadian changes of melatonin to the first night time increases in gonadotropin secretion which herald puberty[16].

Nonetheless our results clearly show that the circulating level of melatonin drops significantly in schoolboys before the first physical signs of puberty have become apparent and before the increase in gonadotropins and testosterone which accompanies puberty. Animal experimentation had indicated that melatonin can act on the hypothalamus and pituitary to suppress the release of LH and FSH[6,7]. Melatonin may also act directly on the gonads to suppress ovarian and testicular function[8]. Thus it is possible that the drop in melatonin, which we have observed, is part of the process which initiates the physical and endocrine changes of puberty.

A major unsolved problem is whether the changes in melatonin secretion also occur in young girls. We have been unable to show any fall in the concentration of melatonin in our schoolgirl population and this may indicate that there is a sex difference in pineal endocrinology. Alternatively there may be an age

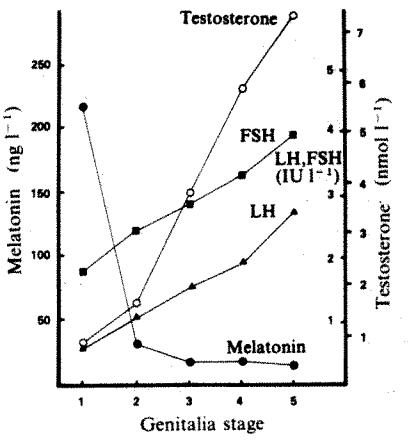


Fig. 2 Mean concentration (calculated from log transformed data) of melatonin (●), FSH (■), LH (▲), and testosterone (○) in serum samples from schoolboys. The values are grouped for each stage of puberty at which they were obtained. Stages: 1, testes, scrotum and penis the same size and proportion as in early childhood; 2, enlargement of testis and scrotum, change in texture and some reddening of scrotal skin; 3, further growth of penis, mainly in length but some increase in breadth; 4, further enlargement of penis in length and breadth and development of glans; 5, genitalia adult in size and shape.

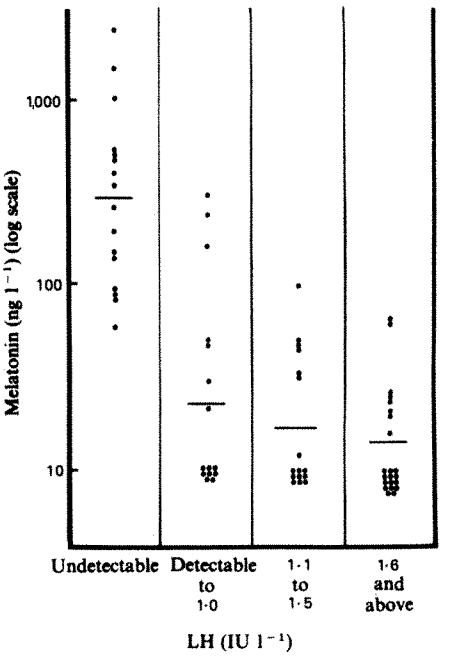


Fig. 3 The concentration of melatonin has been plotted against the concentration of LH in the same sample and the values of LH shown as four groups. *a*, LH undetectable, mean concentration of melatonin 287 pg ml^{-1} (95% confidence limits 494–167, $n = 16$); *b*, LH detectable to 1.0 IU l^{-1}, melatonin 24 pg ml^{-1} (48–12, $n = 15$); *c*, LH 1.1–1.5 IU μl^{-1}, melatonin 17 pg ml^{-1} (24–11, $n = 16$); *d*, LH 1.6 and above IU l^{-1} melatonin 14 pg ml^{-1} (21–10, $n = 22$). The mean concentration of melatonin in each group is shown by the horizontal bar. The limit of detection for the GCMS assay of melatonin was approximately 10 pg ml^{-1}.

difference insofar as the girls have matured earlier than the boys. Thus a drop in the concentration of melatonin may have occurred in the girls but at a younger age than we were able to obtain in our school sample.

Finally, it is curious that the fall in concentration of melatonin is probably evident without the need to use expensive technology. Melatonin is the most potent lightener of melanocytes[17] and has been shown to lighten mammalian hair[18] and human skin[19]. Because children are fairer skinned and lighter haired when they are young and become darker as they grow older, it is likely that the fall in concentration of melatonin is the stimulus for such colour changes, giving visible sign of the early endocrine changes of puberty.

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Specific distribution of metabolic alterations in cerebral cortex following apomorphine administration

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The topographic distribution of dopaminergic receptors in the cerebral cortex closely parallels that of the dopaminergic innervation[1-6]. In the rat, dopaminergic axons which originate in the mesencephalon are confined to a few discrete regions of the neocortex—anterior cingulate cortex, entorhinal cortex, frontal cortex (particularly anteromedial and supragenual areas) and the transitional zone between the neocortex and the pyriform cortex[1-4]. Moreover, biochemical examinations of processes generally considered to be indicative of dopaminergic neurotransmission—neuronal uptake of labelled dopamine or dopamine-activation of adenylate cyclase activity[5,6]—have confirmed a highly restricted locus of action of dopaminergic systems in the cerebral cortex. We describe here data obtained using the 2-deoxyglucose technique[7] in conjunction with conventional neuropharmacological techniques, suggesting that the influence of dopaminergic systems on cortical function extends beyond the known confines of the mesocortical dopaminergic system.

The technique using ^{14}C-labelled 2-deoxyglucose for the measurement of the rates of local cerebral glucose utilisation[7] offers a novel approach to study the pharmacology and physiology of the central nervous system (CNS). The energy requirements of cerebral tissue are derived mainly from the metabolism of glucose, and alterations in functional activity of any region of the brain are associated with changes in its energy demands[8]. The ^{14}C-labelled 2-deoxyglucose technique, which involves the use of quantitative autoradiography to localise radioactivity in the CNS, makes possible the comprehensive assessment of functional alterations throughout the CNS with a degree of localisation impossible with many other approaches[8]. Details of this method have been reported previously[7]. Our experiments were performed on conscious, restrained, male Sprague–Dawley rats weighing approximately 350 g, in which a femoral artery and vein had been catheterised at least 2 h previously. Local rates of glucose utilisation were calculated, by means of the operational equation of the method[7], from the local concentrations of ^{14}C in cerebral tissue (determined by quantitative autoradiography) and the time course of the arterial plasma ^{14}C-deoxyglucose and glucose concentrations. Drugs were administered intravenously; apomorphine was injected 10 min and haloperidol 25 min before the pulse of ^{14}C-deoxyglucose. Administration of the drugs was associated with gross behavioural alterations. With apomorphine, the pattern was characterised by sniffing, licking and movement of the upper body, all of which were replaced at higher concentrations by almost continuous gnawing. With haloperidol, all animals displayed lesser degrees of spontaneous movement, culminating, at doses of 0.1 mg per kg or greater, in a dose-dependent cataleptic state.

Administration of apomorphine resulted in statistically significant dose-dependent alterations in the rates of glucose utilisation in several regions in the CNS in which dopamine is suspected to play a major functional role. For example, glucose utilisation was increased in the caudate nucleus by 29%, in the globus pallidus by 41%, in the substantia nigra zona compacta by 41%, and in the substantia nigra zona reticulata by 76% after the administration of apomorphine (0.5 mg per kg). The effects of apomorphine in the extrapyramidal and other systems will be described in detail elsewhere. We report here only the effects observed in the cerebral cortex, which suggest that the action of apomorphine, the most widely used dopaminergic receptor agonist, on glucose utilisation in the cerebral cortex of the conscious rat is widespread and complex, in spite of the limited extent of cerebral cortical dopaminergic receptors and innervations.

The rate of glucose utilisation normally varies relatively little in different areas of the cerebral cortex, except in the primary auditory cortex where it is markedly greater than in other cortical regions[7]. Metabolic activity is also fairly uniform throughout the depths of the cortex, with the exception of cortical layer IV in which glucose utilisation is approximately 50% greater than in other cortical laminae (Figs 1 and 2).

Apomorphine effected dose-dependent reductions of glucose utilisation in the anterior cingulate cortex, a region innervated by dopaminergic neurones[1-4] and possessing dopaminergic receptor mechanisms[5] (Fig. 2*a*). In contrast, in the dorsolateral frontal cortex and the sensory motor cortex, regions not generally recognised as primary dopaminergic projection areas, apomorphine caused significant increases in glucose utilisation (Fig. 2*b*). In the frontal and sensory motor cortices, the most marked elevations of glucose utilisation were observed in the laminate corresponding to layer VI and the deeper portions of layer V (Fig. 2*c*). Thus, in apomorphine-treated animals this layer, in addition to layer IV, could readily be distinguished on the autoradiographs (Fig. 1). Quantitative determination of the rates of glucose utilisation confirmed the changes seen in the autoradiographs (Fig. 2). Statistically significant increases in the sensory motor cortex were observed even with the smallest dose of apomorphine (0.15 mg per kg) used (Fig. 2). In addition to this laminar pattern of metabolic activation in the dorsolateral

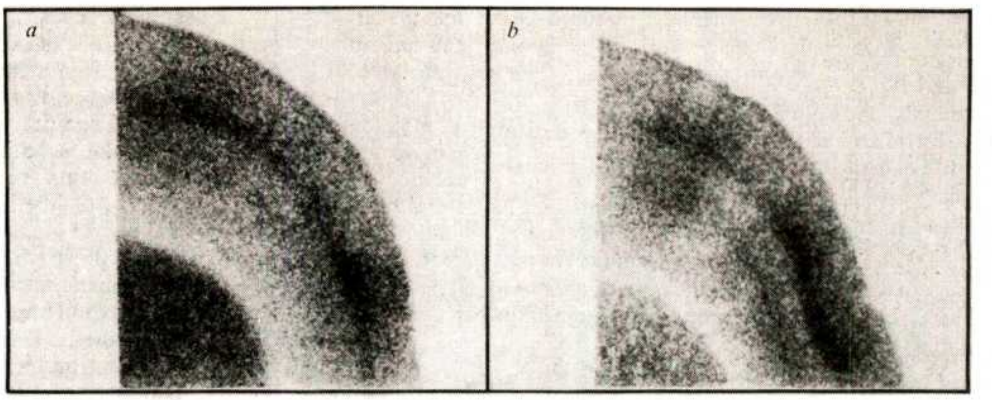


Fig. 1 Autoradiograms of brain sections illustrating the action of apomorphine on the pattern of glucose utilisation in sensory motor cortex of a control animal (*a*) and an animal which had received apomorphine (*b*) (1.5 mg per kg).

frontal and sensory motor cortices, alternating columns of increased and decreased glucose utilisation traversing the thickness of the cortex in these same regions were readily discernible in the autoradiographs (Fig. 1). The columnar and laminar alterations in cortical glucose utilisation decreased progressively in a rostro-caudal direction. In a number of major cortical regions, such as the posterior parietal cortex (Fig. 2*d*), the primary visual cortex and the primary auditory cortex, no alterations in metabolic activity were observed after administration of apomorphine. All the effects of apomorphine in all the cortical areas examined could be prevented by prior administration of haloperidol (0.1 mg per kg).

Previous investigations of the influence of dopamine-agonists and antagonists on cortical function have concentrated on the mesocortical dopaminergic system (particularly, the anterior cingulate cortex and the anteromedial frontal cortex). It is within these small cortical regions that: (1) the dopaminergic nerve terminals originating from the A9 and A10 cell groups of the mesencephalon are found[1–4]; (2) dopamine-sensitive adenyl cyclase has been characterised[5]; (3) specific uptake mechanisms for dopamine have been identified[6]; and (4) cell groupings in which the firing rate is sensitive primarily to dopamine have been localised[9]. Thus, in view of the close coupling between local functional activity and glucose utilisation in the CNS[8], the observation that glucose utilisation in the anterior cingulate cortex is sensitive to a dopaminergic agonist, such as apomorphine, is not unexpected. The marked metabolic activation in the frontal cortex and sensory motor cortex, particularly in the

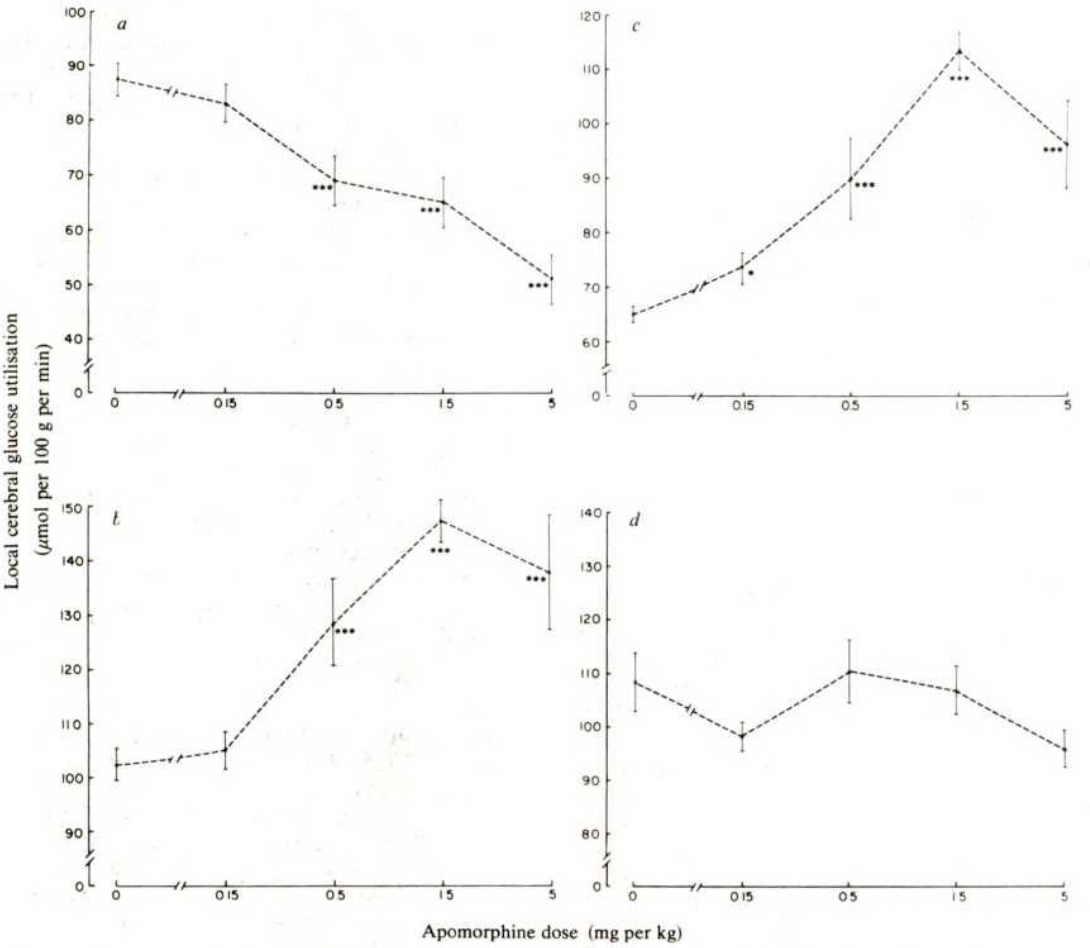


Fig. 2 Log dose–response curve for the rate of glucose utilisation in various cortical areas after intravenous injection of apomorphine. *a*, Anterior cingulate cortex; *b*, sensory motor cortex layer IV; *c*, sensory motor cortex layer VI; *d*, posterior parietal cortex. Data are from 25 animals and are presented as mean ± s.e.m.
$^{*}P < 0.05$. $^{***}P < 0.001$.

deeper laminae, after administration of apomorphine, is of considerably greater interest. It is noteworthy that it is in layer VI and the deepest portion of layer V that the dopaminergic terminals[4] and dopamine-sensitive cells[9] have been identified in the rostral area of the medial aspect of the frontal cortex. There is little evidence, however, of a similar dopaminergic system in layer VI of the dorsolateral frontal cortex and the sensory motor cortex. The regions of cortex in which glucose utilisation increased after administration of apomorphine correspond closely with those areas to which the ventral nucleus of the thalamus projects[10,11]. Glucose utilisation in this thalamic nucleus was increased after administration of apomorphine, and the metabolic activation observed in the dorsolatral frontal and sensory motor cortices may reflect, at least in part, increased activity of non-dopaminergic thalamocortical systems.

The interpretation of the results of any neuropharmacological investigation depends on the specificity of action of the agents used. In the study reported here, the sensitivity to apomorphine of glucose utilisation in areas such as layer VI of the sensory motor cortex, and the prevention of the apomorphine-induced alterations in glucose utilisation in all cortical areas by low doses of the dopamine-antagonist, haloperidol, suggest a key involvement of dopaminergic receptors in the responses observed. Our finding that the cortical involvement in the action of apomorphine extends beyond the known confines of the mesocortical dopaminergic system suggests a need for reappraisal of the mechanisms and foci of action underlying the behavioural effects of antipsychotic and other drugs, which are used to manipulate dopaminergic systems in the CNS.

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Growth and differentiation of aggregating fetal brain cells in a serum-free defined medium

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Aggregating cultures of mechanically dissociated fetal brain cells provide an excellent system for neurobiological studies of cellular growth and differentiation[1–5], but, in common with almost all culture systems, they have the disadvantage that crude serum is required in the medium. Although several cell lines have either been adapted to serum-free conditions[6–12] or grown normally in serum-free media supplemented with hormones, trace elements and defined serum components[12–16], this approach has never been applied to differentiating primary cells of the central nervous system. We now describe the successful cultivation of aggregating fetal rat brain cells in a chemically defined, serum-free medium.

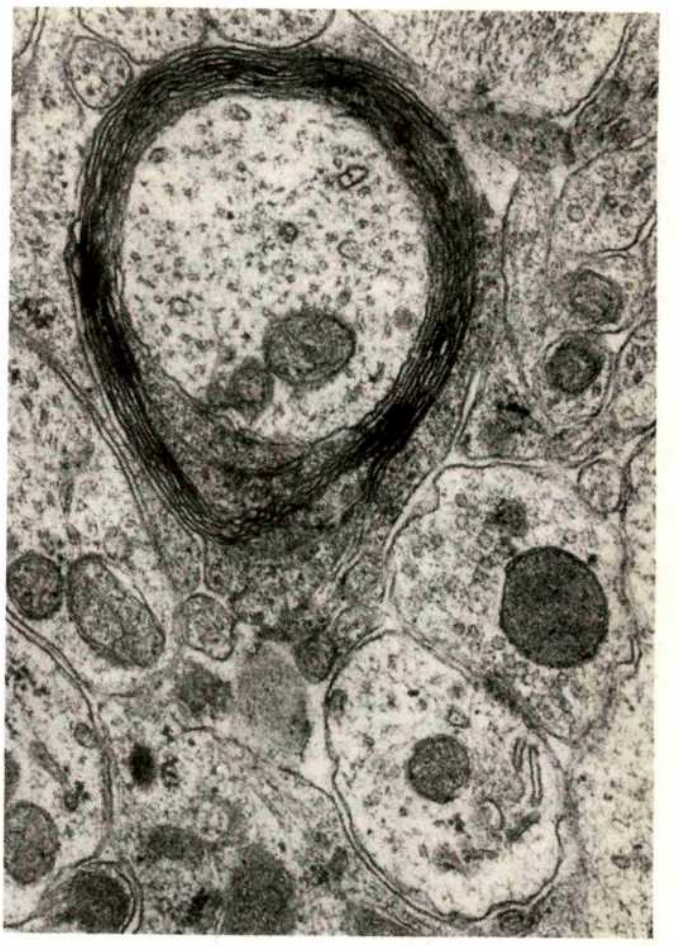


Fig. 1 Electron micrograph of a 35-d aggregate grown in S^- medium, showing a myelinated axon and a synaptic contact. The tissue was fixed in paraformaldehyde–glutaraldehyde, postfixed in osmium tetroxide and embedded in Epon. Sections were stained with uranyl acetate and lead citrate (×42,000).

Equal numbers of mechanically dissociated fetal (15–16 d gestation) rat brain cells[1,2] were inoculated either in Dulbecco–Vogt modified Eagle's medium (DMEM) containing fetal calf serum, referred to as S^+ medium, or in serum-free DMEM supplemented with insulin (5 $\mu g\,ml^{-1}$), 20 nM hydrocortisone, 0.3 nM triiodothyronine, transferrin 1 $\mu g\,ml^{-1}$ and trace elements (see Table 1 legend), referred to as S^- medium. In either case the dissociated cells re-aggregated readily to give a uniform population of aggregates. Throughout the culture period, aggregates in S^- medium remained somewhat smaller, but more numerous (two or threefold) than those in S^+ medium.

Cell proliferation was examined by measuring both the total DNA content and the incorporation of ^{3}H-thymidine into a trichloroacetic acid (TCA)-precipitable macromolecular fraction (Table 1). Cultures grown in S^- medium (S^- cultures) had a greater DNA content and a smaller protein/DNA ratio than cultures grown in S^+ medium (S^+ cultures). In addition, cell counts of thionine-stained semi-thin sections of aggregates revealed almost twice as many cells per unit area in S^- than did S^+ cultures. Both observations suggest slower cell growth (for example, protein acquisition) in S^- cultures. Incorporation of ^{3}H-thymidine (representing DNA synthesis) was demonstrated in both S^+ and S^- cultures (Table 1). During the first 10 d of culture, S^- cultures showed a higher rate of DNA synthesis, with maximum activity after about 5 d. After 10 d, DNA synthesis diminished more rapidly in S^- than in S^+ cultures and by day 20 it was only about one-third of that of S^+ cultures. Lack of either insulin or transferrin in S^- medium resulted in a reduction of both total DNA and ^{3}H-thymidine incorporation at day 5, whereas no difference was observed in the initial rate of ^{3}H-thymidine uptake between cultures grown either in the presence

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or absence of insulin. These results suggest that both insulin and transferrin stimulate cell proliferation in S^- cultures, and this agrees with results of investigations with established cell cultures grown in serum-free conditions[12,14,16]. Lack of either hydrocortisone or triiodothyronine, two other hormones that stimulate the growth of some cell lines[14], had no apparent effect on DNA synthesis.

The morphological differentiation of the aggregates was followed by electron microscopy. Compared with S^+ cultures, S^- cultures underwent generally slower maturation of neuronal processes and delayed myelin synthesis. In S^- cultures no synaptic profiles were found after 4 d, but they were frequent after 8 d; the first myelinated axons appeared at about day 30. After 35 d numerous synaptic contacts and many myelinated fibres were observed (Fig. 1). The compaction of myelin sheaths often appeared incomplete.

The biochemical differentiation of the cultures was studied at regular intervals by measuring the specific activities of the following neurotransmitter metabolising enzymes: choline acetyltransferase (CAT, EC 2.3.1.6), acetylcholinesterase (ACE, EC 3.1.1.7), glutamate decarboxylase (GAD, EC 4.1.1.15), aromatic L-amino acid decarboxylase (AAD, EC 4.1.1.26) and monoamine oxidase (MAO, EC 1.4.3.4). Table 2 shows that in S^- cultures, the specific activities of all enzymes (and the protein content) increased considerably during the first month. In contrast to S^+ cultures, the phase of rapid increase in enzyme specific activities occurred later and no plateau was reached within 4 weeks (with the exception of MAO). Compared with S^+ cultures, aggregates grown for 33 d in S^- had higher specific activities of GAD (192%), AAD (148%) and ACE (168%), but lower CAT (40%) and MAO (51%). Lack of insulin in S^- medium resulted in a significant reduction of both GAD activity and protein content, the reduction of GAD activity being more pronounced at early developmental stages (57% reduction at day 11 compared with 15% reduction at day 33). Lack of transferrin in S^- caused a progressive decrease in protein content and the specific activities of all enzymes except MAO. On the other hand, lack of hydrocortisone or triiodothyronine had no apparent effect on the enzyme activities. In view of the relatively high GAD activity in S^- cultures and because the possible neuronal localisation of this enzyme required verification[20,21], we examined the formation of labelled γ-aminobutyric acid (GABA) from L-[U-^{14}C]glutamate in aggregates (Fig. 2). Compared with S^+ cultures, S^- cultures took up glutamate at a higher rate (Fig. 2*a*) and had an almost two-fold greater net synthesis of GABA (Fig. 2*b*), the latter finding agreeing with the differences in GAD activity measured in homogenates (Table 2). The formation of GABA from labelled glutamate was measured in the presence of the carbonyl-trapping agent aminooxyacetic acid (AOAA). After 30 min of incubation in the presence of 13 μM AOAA, synthesis in both S^+ and S^- aggregates of ^{14}C-GABA was less than 5% of that in control cultures. This contrasts with studies of astrocyte cultures[21], in which 13 μM AOAA did not inhibit GABA formation. Furthermore, both GAD activity and rate of GABA synthesis were higher in aggregates than in astrocytes[21] by at least an order of magnitude. Thus the GAD activity measured in aggregates probably represents the neuronal enzyme activity.

The accumulation of glutamine formed from labelled glutamate was considerably lower in S^- than in S^+ cultures (Fig. 2*c*). Glutamine synthetase (EC 6.3.1.2), the enzyme involved in the amidation of glutamate, has been shown to be localised in glial cells[22], suggesting that S^- cultures contained relatively fewer glial cells than S^+ cultures. This is corroborated by the relatively low specific activity of MAO in S^- cultures. (Several observations suggest that the MAO activity of neuronal cells in culture is considerably lower than that of glia—ref. 23 and P.H., unpublished.) Thus, a proportionately higher number of neurones in S^- aggregates could fully account for the relatively

Table 1 DNA synthesis in aggregating cultures of fetal rat brain cells

Condition	Days in culture	TCA precipitable radioactivity (10^{-6} × d.p.m. per flask)	Total DNA (μg per flask)	Total protein (mg per flask)	Protein/DNA
S^+	2	1.2	126	3.0	24
	5	3.6	155	4.0	26
	10	3.2	120	6.1	50
S^-, complete	2	3.9	316	4.0	13
	5	12.3	383	4.9	13
	10	6.0	270	7.0	26
S^-, no insulin	5	4.0	265	3.4	13
S^-, no transferrin	5	2.2	293	3.9	13
S^-, no hydrocortisone	5	11.1	356	4.6	13
S^-, no triiodothyronine	5	12.1	360	4.8	13

Brains of fetal (15–16 d gestation) Wistar rats (Madörin, Füllinsdorf) were dissected and dissociated mechanically as before[1,2]. The cells were washed three times with Puck's D_1 solution by trituration and centrifugation (3,700g_{av} per min), and resuspended in serum-free Dulbecco–Vogt modification of Eagle's medium (DMEM, high glucose, no pyruvate, GIBCO no. 320–1965, supplemented with: vitamin B_{12} 1.36 μg ml^{-1}, Sigma; biotin 0.007 μg ml^{-1}, Sigma; DL-α-tocopherol 10 μg ml^{-1}, Sigma; retinol 5 μg ml^{-1}, Fluka; lipoic acid 0.2 μg ml^{-1}, Sigma; linoleic acid 0.1 μg ml^{-1}, Sigma; penicillin 50 U ml^{-1}, ICN; and streptomycin sulphate 50 μg ml^{-1}, ICN). This single cell suspension of 3×10^7 viable cells per ml was divided into two equal portions and then diluted with 2 volumes of either S^+ medium (DMEM containing 15% (v/v) fetal calf serum (Seromed) or S^- medium (DMEM without serum). The complete serum-free medium (S^-) contained the following additional supplements: crystalline bovine insulin (5 μg ml^{-1}, Sigma) hydrocortisone-21-phosphate (20 nM, Sigma), 3,3',5-triiodo-L-thyronine (0.3 nM, Sigma), human transferrin (1 μg ml^{-1}, Sigma) and various trace elements as listed by Hutchings and Sato[15]. Samples (3.5 ml) of the cell suspensions were placed into 25-ml De Long flasks (Bellco) and incubated at 37 °C in an atmosphere of 10% CO_2/90% humidified air, under constant rotation at 70 r.p.m. (Infors gyratory shaker). After 2 d, the cultures were transferred to 50-ml De Long flasks (Bellco) and 5-ml samples of new media were added. Media were changed (exchange of 5 ml medium) every 3 d. Within the first week of culturing, speed of rotation was increased gradually to 80 r.p.m. DNA synthesis was determined by measuring incorporation of ^{3}H-thymidine into a TCA-precipitable macromolecular fraction[17]. Cultures were incubated for 22 h in normal conditions with [Me-^{3}H]thymidine (Amersham, 46 Ci mmol^{-1} specific activity) to give a final concentration of 25 nM (1.2 Ci ml^{-1}). Controls were incubated for 22 h at 4 °C. Aggregates were then washed three times with solution D_1, homogenised in 0.6 ml of 0.05% (v/v) Triton X-100 using a glass–Teflon homogeniser and sonicated briefly (Branson model B-12, 3 × 3 s at 10 W). Samples (25–100 μl) of the homogenate were mixed with 1.5 ml of cold 10% (w/v) TCA, kept at 0 °C for 15 min and then collected by suction onto GF/A glass fibre filter disks. The filters were washed three times with 5-ml samples of 10% TCA, transferred to glass counting vials and incubated for 12 h in 0.5 ml NCS tissue solubiliser (Amersham). After neutralisation of the digest with glacial acetic acid, 10 ml scintillation cocktail (toluene, containing 6 g l^{-1} of 2,5-diphenyloxazole (PPO) and 75 mg ml^{-1} of 1,4-*bis*-[2-(5-phenyloxazolyl)]-benzene (POPOP)) was added for liquid scintillation counting. Portions of homogenates were assayed for protein by a modification of the method of Lowry *et al.*[18] and for DNA by a modification of the method of Kissane and Robins[19]. The values given are the mean of at least two individual cultures (deviation < 10%). The average radioactivity found in controls (2,600 d.p.m. per mg protein) was subtracted.

Table 2 Development of specific activities of neurotransmitter metabolising enzymes in aggregating cultures of fetal rat brain cells

Condition	Days in culture	Specific enzyme activity (pmol per min per mg protein) CAT	ACE	GAD	AAD	MAO	Total protein (mg per flask)
S^+	11	50	16,100	335	61	320	6.0
	22	225	39,300	776	67	890	8.8
	33	305	45,200	1,137	110	1,325	9.2
S^-, complete	11	11	8,700	376	64	203	5.9
	22	48	26,600	946	91	428	6.8
	33	122	76,100	2,187	163	680	8.8
S^-, no insulin	11	16	5,800	161	42	112	3.4
	22	53	24,600	485	66	381	3.4
	33	148	54,500	1,858	185	666	5.0
S^-, no transferrin	11	7	6,100	307	42	140	5.5
	22	6	13,900	230	40	390	3.9
	33	3	16,100	209	20	670	3.2
S^-, no hydrocortisone	11	8	8,300	330	50	212	5.7
	22	46	24,600	981	76	376	7.1
	33	119	73,600	1,968	131	761	8.5
S^-, no triiodothyronine	11	10	8,200	382	64	212	6.4
	22	41	25,300	869	93	392	6.7
	33	110	69,500	2,050	143	675	8.8

Fetal rat brain cell aggregates were prepared and cultivated in DMEM containing 15% (v/v) fetal calf serum (S^+) or in serum-free DMEM (S^-), as described in Table 1 legend. The methods used for homogenate preparation and enzyme assays have been described before[1]. Protein was determined by a modification of the method of Lowry *et al.*[18], using bovine serum albumin as a standard. The values given represent the mean of at least five individual culture flasks (s.e.m. < 5%).

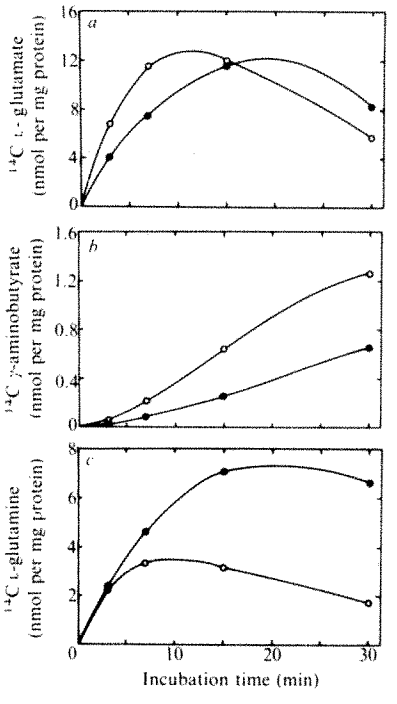


Fig. 2 Aggregates cultured for 30 d in either S^+ medium (●) or in S^- medium (○) were preincubated for 30 min in serum-free DMEM deficient in L-glutamine and subsequently incubated in the presence of 100 μM L-[U-^{14}C]glutamate (Amersham, 1.4 mCi mmol^{-1} final specific activity). The commercially available radioactive material was purified before use by TLC on cellulose plates. The solvent system was 1-butanol/glacial acetic acid/water: 12/3/5 (v/v). After various times, aggregates were analysed for their content of L-^{14}C-glutamate (*a*), ^{14}C-GABA (*b*) and L-^{14}C-glutamine (*c*). The labelled compounds were extracted from homogenates of the washed aggregates and then quantitatively separated on cellulose plates by using the following two-dimensional separation technique: high voltage electrophoresis (buffer: 0.125 M pyridine, 1.05 M acetic acid, *p*H 3.8), followed by TLC (solvent system as above). Further technical details are given in ref. 5. Quantities of products were calculated on the assumption that the specific activity of the precursor in the tissue was equal to that in the medium. Aliquots of the homogenates were assayed for protein by a modification of the method of Lowry *et al.*[18] (s.e.m. < 5%).

high GAD activity observed in these cultures. If this is the case, the relatively low specific activity of CAT, another neuronal marker enzyme, may be explained if, in S^- cultures, (1) there is a smaller proportion of cholinergic neurones; (2) there is a greater retardation in the development of cholinergic neurones; or (3) CAT is more retarded in its development than GAD. Although the present results do not enable us to distinguish between these possibilities, there is some circumstantial evidence in favour of points (2) and/or (3), that is, compared with S^- cultures of cells from the mesencephalon–diencephalon–rhombencephalon region, S^- cultures of telencephalic cells (which are presumably at an earlier stage of development) show a much higher rate of DNA synthesis, a much more pronounced delay of CAT and some delay of GAD maturation (data not shown).

In conclusion, our morphological and biochemical data demonstrate that mechanically dissociated fetal rat brain cells re-aggregate, grow and differentiate in a chemically defined, serum-free medium. Such cultures show some retardation in cellular growth and differentiation compared with their counterparts grown in the presence of 15% fetal calf serum. Although more work is needed to define their developmental characteristics further, serum-free re-aggregating brain cell cultures will be valuable for the study of nutritional and hormonal influences on brain development.

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Androgen receptors exist throughout the 'critical period' of brain sexual differentiation

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Several studies suggest direct roles for androgens and oestrogens in the development of sexually dimorphic characteristics of mouse and rat brain[1]. To elucidate the biochemical mechanisms for such effects, investigators have asked which putative steroid receptors are present in the hypothalamus throughout the critical period of sexual differentiation. *A priori*, potential receptors would include high-affinity proteins with selectivities for active androgens, oestrogens and their metabolites. Two major classes of steroid that might be active as agonists are the androgens *per se*, including testosterone and dihydrotestosterone (DHT)[2], and the oestrogens, which are themselves androgen metabolites[3]. Normally, in sexual differentiation, a proper balance of both androgens and oestrogens may be necessary[4]. Indeed, receptors for each of these ligands do exist in the hypothalamus of neonatal and prepubertal mice[4–7]. Although the perinatal oestrogen receptor and its function have been extensively studied[7,8], the existence of perinatal androgen receptors has not been as clearly demonstrated to permit a similar acceptance[9]. In this report, we establish the existence of adult-like androgen receptors in embryonic and neonatal mouse and rat hypothalami by qualitative biochemical and genetic analyses. This is achieved by DNA–cellulose affinity chromatography and velocity sedimentation, and by analysis of the androgen-resistant mutant, testicular feminisation. The presence of sex hormone receptors in perinatal brain is discussed in the context of behavioural responses which are differentiated during the critical period of brain sexual development.

We used DNA–cellulose affinity chromatography to characterise the androgen-binding proteins in perinatal hypothalamus as it fractionates low levels of androgen receptor in prepubertal brain[10,11] and permits qualitative analysis of the DNA-adhering material[7,8]. Figure 1 shows a representative elution profile of prepubertal hypothalamic androgen receptor (triangles), and also illustrates (circles) a ^{3}H-DHT-binding activity from neonatal hypothalamus which similarly adheres to DNA–cellulose and elutes in the same manner with a linear concentration gradient of NaCl. The androgen-binding activities in both neonatal and prepubertal cytosols exhibit elution maxima in the 140–150-mM NaCl range of the gradient. Therefore, by the criteria of DNA adherence, the androgen-binding activity in perinatal mouse hypothalamus is qualitatively similar to the putative androgen receptor present in older animals.

To determine its macromolecular integrity, the material which elutes from DNA–cellulose was analysed by velocity sedimentation (Fig. 2). As indicated by the lack of radioactivity at or near the top of sucrose gradients following centrifugation, the androgen is indeed bound to macromolecular components of cytosols from prepubertal (21 days after birth; Fig. 2*a*), neonatal (3–4 days after birth; Fig. 2*b*, closed circles) and embryonic (3 days before birth; Fig. 2*b*, open circles) hypothalami. The androgen-binding activities from both neonatal and embryonic hypothalami (Fig. 2*b*) behave similarly to that from prepubertal hypothalamus (Fig. 2*a*) in that all sediment as 4S macromolecules. The sedimentation profiles shown in Fig. 2*a* and *b* are representative of data obtained at all hormone concentrations tested.

Using both DNA–cellulose chromatography and velocity sedimentation, we have assessed the saturability of the androgen-binding activities in embryonic, neonatal and prepubertal tissues. Androgen-binding activities are $92 \pm 3\%$ (s.e.m. $n = 15$) saturated at hormone concentrations below 10 nM; this is observed with both DHT and testosterone in both hypothalamic and kidney cytosols, and agrees with previous observations that putative androgen receptor from prepubertal animals saturates at hormone concentrations between 4 and 8 nM (refs 4, 7).

In mature animals with the androgen-resistant genetic syndrome, testicular feminisation (*Tfm*), the levels of androgen receptor are lower in both hypothalamus[4,10–12] and kidney[7,13–15] than in wild-type animals. As the wild-type neonatal androgen-binding activity which adheres to DNA–cellulose seems to be similar to that of older animals (Figs 1, 2), we would expect this activity in neonatal *Tfm*/Y tissues to be similarly affected if the mutation is expressed at an early age. Indeed, the androgen binding capacity of neonatal *Tfm*/Y cytosols (hypothalamus and kidney) is approximately 15% that of sibling (male and female) cytosols (Table 1); a similar observation has been made in submandibular gland and kidney cytosols using sedimentation analysis[16]. In contrast, the concentration of oestrogen receptor in mouse *Tfm*/Y hypothalamus is similar to that in sibling hypothalamus at the neonatal ages tested (data not shown), as was shown for older animals[4,10].

Thus, our data suggest that both embryonic and neonatal hypothalamus contain an androgen-binding activity which is qualitatively similar to the putative androgen receptor in the hypothalamus of older animals. However, the level of neonatal binding is lower than prepubertal binding (Figs 1, 2).

Using the qualitative DNA–cellulose and velocity sedimentation patterns presented above, we have also quantified the putative androgen receptor content of the developing mouse hypothalamus. These data are summarised in Fig. 3. The concentration of androgen receptor detected in mouse hypothalamus between embryonic and prepubertal ages increases approximately sevenfold. In contrast, between 3 days before and 3 days after birth, only a twofold increase in the concentration of androgen receptor is detected. These data augment earlier quantitative measurements which also indicate an increase between late postnatal and prepubertal ages[6,17]. Thus, the phase of most rapid appearance of androgen receptor seems to coincide with the late phase of the critical period of brain sexual differentiation. This ontogeny differs significantly from that of oestrogen receptors in mouse hypothalamus, as the overall increase in the concentration of oestrogen receptor from embryonic to prepubertal ages is only twofold[7,8]. It is possible that technical or biological factors which differentially affect the detectability of androgen receptors cause this apparent difference. However, in spite of this qualification concerning the levels of androgen receptors, the significance of our report is that it establishes their presence in the hypothalamus throughout perinatal development.

Table 1 Androgen receptors in neonatal *Tfm*/Y and sibling mice

		Testosterone bound (mol × 10^{18} per mg tissue)*	
Age (d)	Genotype	Hypothalamic–preoptic area	Kidney
5	♀	6.7	42.0
	Tfm/Y	1.1	3.9
7	♀	7.0	50.0
	♂	7.5	64.0
	Tfm/Y	0.9	5.5

* Quantification was by DNA–cellulose affinity chromatography, using a protocol similar to that described in Fig. 1 legend.

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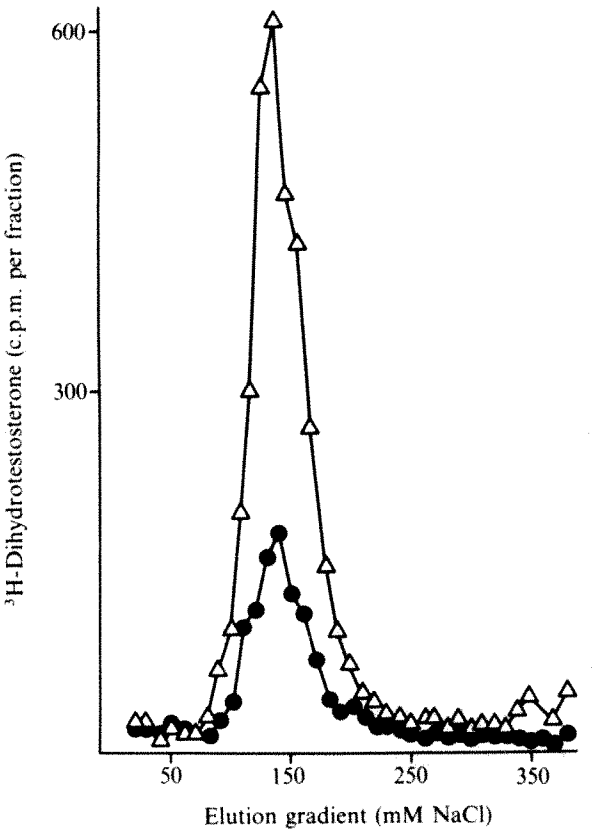


Fig. 1 DNA–cellulose affinity chromatography of androgen receptor from cytosols of neonatal and prepubertal mouse hypothalamus. C57BL/6J mice were obtained from the timed-pregnancy colony in our department. To prepare cytosol extracts, hypothalamic–preoptic area blocks from either 4- and 5-d-old mice (●) or 20- and 21-d-old mice (△) were homogenised at 2 °C, using a Kontes Teflon/glass homogeniser, in buffer containing: 10 nM Tris-HCl (*p*H 8.1, 21 °C), 1 mM mercaptoethanol, 1 mM Na_3EDTA, 10% glycerol and 50 mM NaCl. After the homogenate had been centrifuged at 140,000 *g* for 60 min, the resulting cytosol was collected and incubated with 10 nM [1,2,4,5,6,7-^{3}H(*N*)]5α-dihydrotestosterone (DHT; 152 Ci mmol^{-1}, NEN). Before use, we determined the purity of radioactively labelled steroid by HPLC (high-performance liquid chromatography: Waters Associates Inc.) or TLC (thin-layer chromatography); it was necessary to use purified ^{3}H-DHT which was obtained by HPLC. Following a 60-min incubation with ^{3}H-DHT, the cytosol preparations (each containing an equivalent of 970 mg tissue) were loaded on columns containing 5 ml DNA–cellulose which had been equilibrated with buffer containing 50 mM NaCl. The columns were then washed with approximately 60 ml of buffer during a 12-h period. The ^{3}H-DHT-bound content of the column was eluted with a 60-ml linear gradient of NaCl, ranging from 50 to 500 mM. For each column, fractions were collected and the NaCl concentrations were determined using a Radiometer conductivity meter. Radioactivity was measured after the addition of 5.0 ml of Omnifluor (NEN)–toluene scintillation fluid.

In rat hypothalami at embryonic, neonatal and prepubertal ages, we have also detected androgen-binding activities which adhere to DNA–cellulose and elute sharply at a NaCl concentration of approximately 140 mM. These elution characteristics are similar to those shown in Fig. 1. Saturable androgen-binding capacities of rat hypothalamic cytosols are, respectively, 5.8 ± 1.4 (2–4 days before birth; $n = 3$), 7.7 ± 0.3 (4 days after birth; $n = 2$) and 37.8 ± 8.4 (21–35 days after birth; $n = 3$) mol × 10^{18} per mg tissue (±s.e.m.). In keeping with the mutant mouse results, the androgen-binding activity in the hypothalamus of the androgen-resistant rat[18] is approximately 20% that of the wild type.

Perinatal administration of DHT masculinises some features of both sexual behaviour in rats[19] and guinea pigs[20], and social play in monkeys[21]. The masculine behavioural potential of both aggressive behaviour in female mice[22,23] and sexual behaviour in female rats[24] is influenced by the *in utero* proximity to males, presumably by androgens from testicular secretions. These *in utero* masculinising effects on sexual behaviour are blocked by flutamide[24], a presumed antagonist of direct androgen action. However, masculinisation can occur fully[25,26], or in part[27], after postnatal administration of presumed inhibitors of androgen conversion to oestrogens.

In addition to masculinisation, perinatal administration of androgens or oestrogens can suppress some features of female sexual behaviour in mice and rats[28]. Moreover, androgens including DHT can defeminise some features of sexual behaviour in hamsters[29]. On the other hand, prenatal exposure to flutamide[30] and 1,4,6-androstatsiene-3,17-dione(ATD)[31], which presumably block direct androgen action and oestrogen formation, respectively, enhance lordotic behaviour in female rats. As female sexual behaviour can be suppressed by both androgens and oestrogens and enhanced by agents which block their direct actions, both hormones may mediate these developmental processes. These experiments suggest that androgen receptors could be involved in some aspects of masculinisation and defeminisation of sexually dimorphic behaviours. The present findings provide one possible molecular mechanism

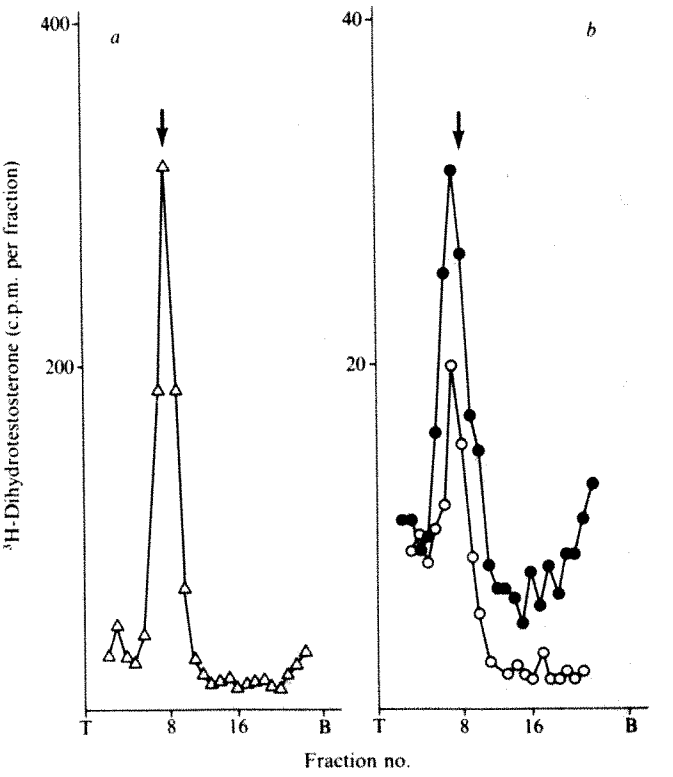


Fig. 2 Velocity sedimentation analysis of androgen receptor from embryonic, neonatal and prepubertal mouse hypothalamus. Hypothalamic blocks from C57BL/6J mice of determined embryonic, neonatal and prepubertal ages were used. Cytosols (containing an equivalent of 300 mg tissue) were prepared, incubated with 20 nM ^{3}H-DHT and chromatographed with DNA–cellulose as described in Fig. 1 legend. The ^{3}H-DHT-bound contents of each column were eluted with a single step of buffer containing 400 mM NaCl and then layered on a sucrose gradient (5 ml; 5% to 20%, w/v) containing the same buffer. Gradients were centrifuged for 20 h at 234,000*g* (average force) and contained the internal fluorescent marker dansyl-BSA (4.7S), the position of which is indicated by the arrow. *a*, Prepubertal cytosols (21 days after birth), △----△. *b*, Embryonic cytosols (3 days before birth), ○----○; neonatal cytosols (4 and 5 days after birth), ●----●.

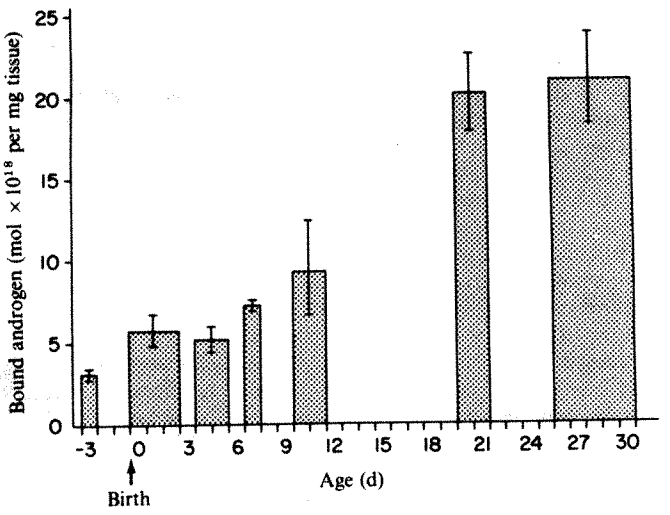


Fig. 3 Concentration of androgen receptors detected in the developing mouse hypothalamus. The horizontal axis represents time in days, with the distance between two markings representing one 24-h period. The vertical axis represents mol of bound androgen obtained by DNA–cellulose chromatography alone or in combination with velocity sedimentation. Velocity sedimentation analysis following DNA–cellulose chromatography was carried out at ages considered representative of embryonic (3 days before birth), neonatal (4–5 days post-birth) and prepubertal (21 days post-birth) development; the macromolecular material recovered following sedimentation was 95.6 ± 3.9% (s.e.m., $n = 8$) of the DNA–cellulose eluate applied to the sucrose gradient. The hormone-binding activities which are quantified as receptor activities are those which adhere to and eluate from DNA–cellulose in a specific manner. For example, in Fig. 1, the neonatal binding activity which elutes within the 75–225-mM region of the NaCl gradient is quantified by summing all those data points. This type of analysis serves as a qualitative control for the quantitative determinations reported above. Error bars indicate the s.e.m. The number of determinations (n) and the mean number of hypothalami per determination (HPOA) for the ages represented above are: 3 days before birth ($n = 2$, HPOA = 95); 0–2 days post-birth ($n = 2$, HPOA = 32); 4–5 days post-birth ($n = 6$, HPOA = 26); 7 days post-birth ($n = 2$, HPOA = 10); 10–11 days post-birth ($n = 3$, HPOA = 10); 20–21 days post-birth ($n = 6$, HPOA = 11); 25–30 days post-birth ($n = 2$, HPOA = 10). Our assignment system for embryonic ages has been described previously[8]. Records of vaginal plugs and births were used for all the age determinations illustrated above.

through which androgens may act as effectors of sexual differentiation.

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A region of the *Drosophila* genome necessary for CNS development

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Mutations in genes involved in essential aspects of central nervous system development in *Drosophila melanogaster* are expected to be lethal. Thus, when searching for neurogenic mutants attention should be focused on embryonic lethal point mutants[1], for many of these might affect neural development. However, this approach can be very time consuming, for the location of neurogenic genes is unknown. A more convenient approach, which allows a faster screening of the genome, is to use relatively small chromosome deletions[2,3] to determine whether the lack of a definite part of the genome affects neurogenesis. Once any region producing an interesting neural phenotype is found, it can be further analysed by the use of smaller deletions or point lethal mutants mapping within it, until the gene(s) responsible can be more precisely localised[1,4]. We report here on a region of the *Drosophila* genome which has been found necessary for normal neurogenesis.

Using the technique designed by Zalokar and Erk[5] for staining embryos as whole mounts, we have studied the effect on embryogenesis of deficiencies (a total of 52) covering ~1,000 bands distributed throughout all major chromosomes of *Drosophila*. We have found that some of them, even large ones, have no effect on the overall morphological pattern of the embryo, whereas others produce rather general morphogenetic defects. We intend to describe these defects in detail elsewhere. In addition to those deficiencies, however, we have found that some at the tip of the first chromosome exclusively alter the morphology of the embryonic nervous system in hemizygous animals, almost entirely eliminating neural tissue without modifying that of the remaining organs. Part of the chromosome region responsible for this abnormal phenotype is the *achaete-scute* system, which it has recently been suggested participates in neural development[6,7]. We have examined the neural phenotype produced by terminal deficiencies of increasing size—*Df(1)sc*^{8}, *Df(1)sc*^{H}, *Df(1)260-1*, *Df(1)y*^{74k}, *Df(1)svr* and *Df(1)Bld*— as well as some intercalary ones—*In(1)sc*^{4L}*sc*^{9R}, *Df(1)sc*^{19}—with the following results (see Fig. 1). *Df(1)sc*^{8}, the smallest terminal deficiency studied, does not seem to change the pattern of the nervous system observed in wild-type embryos. However, all remaining deletions eliminate increasing amounts of neural tissue, the extent of the lesion being correlated with the size of the chromosome deletion considered. The smallest intercalary deficiency studied (*In(1)sc*^{4L}*sc*^{9R}) only produces a main constriction in the middle of the ventral cord, whereas embryos hemizygous for the largest terminal deficien-

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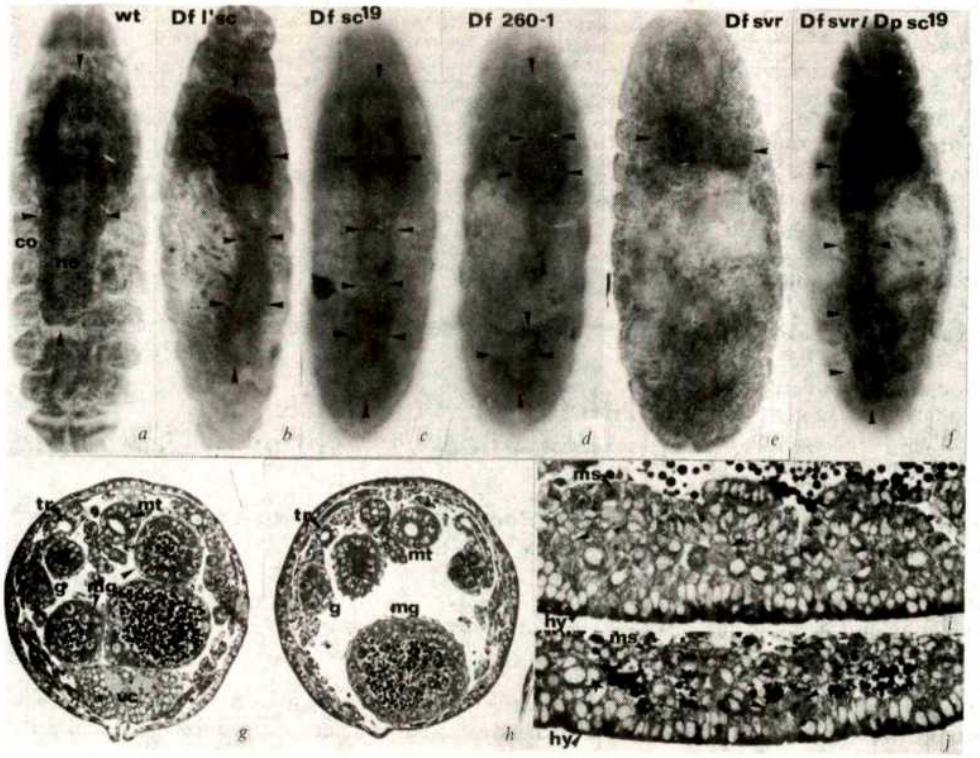


Fig. 1 Neural phenotype found in embryos hemizygous for deficiencies at the tip of the first chromosome. The experimental crosses were made with F_1 females, derived from an outcross of the deficiency-carrying stock to a wild-type (Oregon S) stock and wild-type males. The only exception was *Df(1)svr-Dp(1)sc*^{19}, in which $\widehat{XX}$/ *Y; Dp(1)sc*^{19}/*CyO* females were crossed with *Df(1)svr/ y*^{2}*Y67g* males. Embryos collected at 17 ± 1 (*a–h*) and 7 ± 0.5 (i, j) h after oviposition (25 °C) were fixed either with alcohol/acetic acid/formaldehyde for fuchsin staining of whole mounts or with glutaraldehyde–osmium tetroxide for Durcupan embedding for light and electron microscopy[5]. *a* Shows a whole mount of a wild-type embryo. *Df(l'sc (b)*, corresponding to *In(1)sc*^{4L}*sc*^{9R} which includes the lethal of *scute* (*l'sc*)[7,11], produces a constriction in the abdominal ganglia in the ventral cord, which has not completely undergone the condensation normally occurring at this time[8] (16–18 h of development). Constrictions and lack of condensation become more evident in *Df(1)sc*^{19} *(c)* or in *Df(1)sc*^{H} (not shown) embryos, both deficiencies producing a comparable phenotype. *d* Is an example of *Df(1)260-1* embryos, showing a large gap between rostral and caudal parts of the ventral cord. *e* Illustrates the phenotype found in *Df(1)svr* embryos, in which only remnants of the brain lobes are visible. *f* Shows the result of covering the left part of the region of interest with the *Dp(1; 2)sc*^{19}; the phenotype obtained is similar to that of the deficiency for the *achaete-scute* system *Df(1)sc*^{19} shown in *c*. Photographs were taken focusing the suboesophageal ganglion and the ventral cord so that the brain lobes are not clearly defined. The arrowheads mark the borers of the nervous system, co, cortex; ne, neuropile. *g, h*, Transverse sections at the level of the gonads through wild-type (g) and *Df(1)y*^{74k} (*h*) embryos. g, gonads; mg, midgut; mt, malphighian tubules; tr, tracheae; vc, ventral cord. *i, j* Sagittal sections through the ventral region of wild-type (*i*) and *DF(1)svr* (*j*) 7-h-old embryos. Dividing neuroblasts[9] (arrowheads) in both instances and clusters of degenerating cells (asterisks) in the mutant are evident. hy, hypodermis; ms, mesoderm.

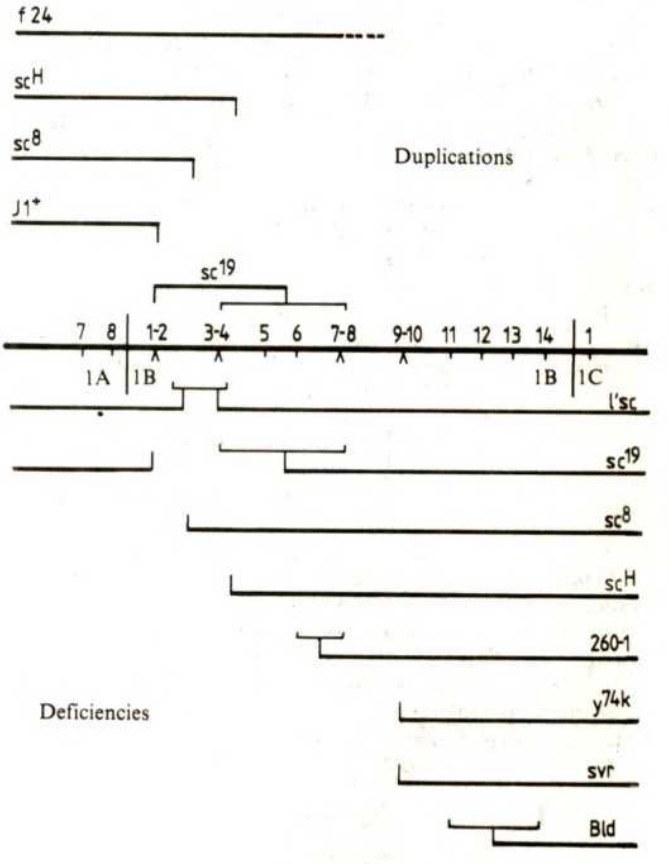


Fig. 2 Cytogenic location and extent of deficiencies and duplications used in the present work. In the centre of the figure, bands within subdivision 1B of the X chromosome are given according to Bridges' revised map[12]. The lower half of the figure shows the deficiencies studied. Data of breakpoints are taken from refs 7, 10, 11 and 13. Some uncertainties exist concerning the location of deficiency breakpoints. For example, in *Df(1)l'sc*, corresponding to *In(1)sc*^{4L}*sc*^{9R}, genetic data[7,14] demonstrate that *In(1)sc*^{9} has a distal breakpoint to the right of that of *In(1)sc*^{4}; cytogenetically, however, the sequence is the inverse[11]. The right breakpoint of *Df(1)sc*^{19} has been found[11] at 1B4-7; genetically (ref. 7 and present work), it should be at the same location as that of *Df(1)sc*^{H}—1B4—as the phenotype produced by both deficiencies is very similar. Finally, the breakpoint of *Df(1)Bld* has been genetically and cytogenetically localised at 1B11-14. The upper half of the figure shows the extent of duplications according to refs 7 and 11 and to data from the present work.

cies (*Df(1)y*^{74k}, *Df(1)svr* and *Df(1)Bld*) do not show any organised ventral cord structure but only some cell clusters in the brain lobes (Fig. 1*a–f*).

We then studied serial sections of mutant embryos, and confirmed the previous observations. Note that in the mutants studied, all other embryonic structures that we are able to recognise on histological sections seem to be entirely normal at 17 ± 1 h of development. This can be seen, for example, in the sections shown in Fig. 1*g* and *h*, corresponding to comparable levels of wild-type and *Df(1)y*^{74k} embryos, where the only appreciable difference is the absence of the ventral cord in the mutant. However, a small but increasing number of dead cells is visible in the hypodermis of developing 10–14-h-old *Df(1)260-1*, *Df(1)svr* and *Df(1)Bld* embryos, although the hypodermis of 17 ± 1-h-old embryos shows no defect in any of these mutants.

The nature of the lesion found at late stages of development was traced back by analysing younger mutant embryos—*Df(1)sc*^{19}, *Df(1)260-1* and *Df(1)svr*. No abnormalities were detected at 4 h, but 7-h-old animals already displayed extensive cell death throughout both brain lobes and ventral cord anlagen (Fig. 1*i*, *j*), the extent being largely dependent on the deficiency considered, and the definitive phenotype being eventually attained at 13 h of development. As dividing neuroblasts seem to be present[8] in the mutant, cell death must chiefly affect ganglion mother cells[9] and/or differentiating neurones. From the above results, we suggest that the part of the genome responsible for this phenotype codes for functions necessary for the development of the nervous system. A more detailed analysis has been made feasible by covering part of the deleted region with corresponding pieces of the wild-type first chromosome translocated into other chromosomes. This analysis has demonstrated that the region to the right of *scute* also affects neural development (see Fig. 2). For example, by covering the left part of the region of interest with duplications, as in *Df(1)svr-Dp(1; 2)sc*^{19} (Fig. 1*f*) or *Df(1)svr-Dp(1; 4)sc*^{H}, a phenotype roughly comparable to that of deletions of the *achaete–scute* system (for example, *Df(1)sc*^{19}, (Fig. 1*c*) or *Df(1)sc*^{H}) is obtained. Thus, the analysis of deficiency–duplication combinations demonstrates that both the *achaete–scute* system and the region to its right might contain qualitatively similar signals.

The left border of the region under study is defined by *Dp(1; Y)l(1)J1*$^{+}$; it is the largest terminal duplication of those we have studied which is unable to rescue the phenotype of either *Df(1)260-1* or *Df(1)svr*. The right border may be tentatively placed in the vicinity of the breakpoint of *Df(1)Bld* (see Fig. 2). This leaves us with a relatively large fraction of the *Drosophila* genome—almost all the subdivision 1B of the first chromosome—apparently coding for functions of related nature, as judged from the resulting phenotype. The problem of how specifically the whole region controls neural functions requires further investigation. Cell death in the developing hypodermis of some mutants, and the fact that *Df(1)svr* has been reported to be inviable in clones of imaginal epidermal cells whereas clones of *Df(1)260-1* cells are viable[10], suggest that some gene functions included in the *Df(1)svr* might also be needed by tissues other than the nervous system. However, *Df(1)svr* is a large deletion, comprising at least 16 complementation groups[10], so that these findings do not necessarily exclude a specific control of nervous system development by genes from this region. Even so, the results described here clearly demonstrate the efficacy of chromosome deletions as a tool with which to screen for neurogenic loci within the *Drosophila* genome.

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Histone gene deficiencies and position–effect variegation in *Drosophila*

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Position–effect variegation, originally observed in insects, has since been demonstrated in plants and mammals. When cells bear a chromosomal rearrangement which juxtaposes a euchromatic gene near to heterochromatin, a fraction of the cells exhibit no expression of that gene. The resultant organism is a mosaic for activity of the rearranged gene. The degree of mosaicism can be modified; such factors as elevated developmental temperature, additional Y chromosome heterochromatin in the genome, and various modifier genes enhance the proportion of cells in which the gene is active[1]. Although the phenomenon has been extensively documented, the underlying molecular mechanism of position–effect variegation remains unclear. The proportion of cells exhibiting gene inactivation increases with greater proximity of the rearranged gene locus to heterochromatin. It has been proposed that the variegating gene is inactivated by a 'spreading effect', or limited spatial diffusion of molecules from the adjacent heterochromatin[2]. This explanation supposes that the juxtaposed gene locus is condensed into a transcriptionally inactive state, possibly by a phosphorylated histone protein subspecies characteristic of *Drosophila* heterochromatin[3]. Such a model leads to the prediction that an alteration in the amount of cellular histone protein would affect the expression of variegating genes. To test this prediction, we have investigated the effect of a reduction in the number of genes coding for histone protein on the degree of mosaicism observed in two variegating gene systems. Our results suggest that the amount of variegating gene expression is a function of histone gene multiplicity.

The histone genes of *Drosophila melanogaster* have been localised to a discrete region on the left arm of the second chromosome (polytene bands 39D3–E2)[4]. This region contains a nucleotide sequence coding for all five histone protein species, tandemly reiterated about 100 times[5]. Individuals whose genotypes contain single or triple histone clusters instead of the normal diploid complement are viable and phenotypically normal. Deficiencies in the left arm of the second chromosome have been generated, and characterised with respect to their breakpoints[6]. Five of these deficiency chromosome stocks were obtained; all have similar breakpoints to the left of the histone gene cluster. Two of the deficiencies, *Df(2L)1* and *Df(2L)12*, extend rightwards towards the histone gene cluster, but do not include it (Fig. 1). They serve as controls for any effect of deleting euchromatic segments to the left of the histone gene cluster. One deficiency, *Df(2L)84*, has its right breakpoint in the

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Table 1 Mean percentage of the wild-type amount of drosopterin in eyes of $In(1)w^{m4}/Y$ F_1 flies from the cross: $+/Y;CyO/Df(2L)\delta\delta \times w^{m4}/w^{m4};+/+\varphi\varphi$

F_1 δ genotype	No. of histone gene clusters	% w^+ drosopterin	S_Y	P
$w^{m4}/Y;Df(2L)1/+$	2	4	1.2	>0.05
$w^{m4}/Y;CyO/+$	2	3	0.7	
$w^{m4}/Y;Df(2L)12/+$	2	4	0.9	>0.05
$w^{m4}/Y;CyO/+$	2	3	0.5	
$w^{m4}/Y;Df(2L)84/+$	1–2	19	2.4	$\ll 0.05$
$w^{m4}/Y;CyO/+$	2	6	0.7	
$w^{m4}/Y;Df(2L)65/+$	1	9	2.1	<0.05
$w^{m4}/Y;CyO/+$	2	3	0.4	
$w^{m4}/Y;Df(2L)161/+$	1	24	3.2	$\ll 0.05$
$w^{m4}/Y;CyO/+$	2	8	1.7	
$w^{m4}/Y;+/+$	2	4	0.9	

F_1 progeny were reared at 17 °C and allowed to age to 1–7 d post-eclosion. Approximately 50 flies of each type were decapitated by freezing and agitation. Single heads were crushed on cellulose chromatography plates (Eastman 13255) and pigments were separated with a 2:1 propanol: 1% NH_3 solution. The drosopterin spots were located, and relative levels of fluorescence measured, using a Zeiss microscope equipped with a UV light source and a photomultiplier. The fluorometer was standardised against the amount of drosopterin fluorescence from w^+ (Oregon-R strain) heads. Significance of difference in mean drosopterin amounts between $Df(2L)/+$ and $CyO/+$ siblings was tested using a t-test.

histone gene cluster. The proportion of the cluster which it deletes is uncertain. The remaining deficiencies, *Df(2L)65* and *Df(2L)161*, delete the entire histone gene cluster. The five deficiencies were tested on two chromosomal rearrangements which exhibit position–effect variegation. The first was $In(1)w^{m4}$, an inversion which relocates the *white*$^+$ gene from its normal position at the tip of the X chromosome to the centromeric heterochromatin. The *white*$^+$ gene is responsible for the formation of pigment granules in the pigment cells[7]. Individual cells of a *white* variegated, mosaic eye have either a normal complement of pigment granules, or are devoid of them[8]. A $w^{m4}/Y;+/+$ eye contains about 4% of the wild-type amount of the red pigment drosopterin, when flies are raised at 17 °C. The control deficiencies on the second chromosome (*Df(2L)1*, *Df(2L)12*) do not significantly alter this level (Table 1). $In(1)w^{m4}$ flies heterozygous for the other three deficiencies which delete all, or a portion of the histone gene cluster, exhibit significantly elevated levels of eye drosopterin. Active expression of the *white*$^+$ gene occurs in a greater proportion of $In(1)w^{m4}$ pigment cells with a single histone gene cluster than in those with the normal diploid complement.

The narrow eye phenotype associated with the mutation *Bar* is the result of a tandem duplication in the X chromosome region 16A (ref. 9). The rearrangement $B^{SV}Y$ attaches this duplicated region to the heterochromatin of the Y chromosome[10]. As this transposed region is inactivated by position–effect variegation in a proportion of the presumptive ommatidial (eye facet) cells, B^{SV} flies exhibit a phenotype intermediate between the narrow eye of *Bar* and the normal, oval *Bar*$^+$ eye. In this case, suppression of the spreading effect should result in a narrower eye phenotype in B^{SV} flies, as the transposed duplication segment would be actively expressed in a greater proportion of presumptive ommatidial cells. This was observed in B^{SV} flies heterozygous for deficiencies of the histone gene cluster (Table 2). The effect of deficiency *Df(2L)84*, which deletes a portion of the histone gene cluster, is not as pronounced as that of deficiencies *Df(2L)65,161* which delete the entire cluster.

The results obtained using these two variegating gene systems confirm the prediction that a reduction in histone gene dosage will enhance active expression of variegating genes. Khesin and Liebovitch[11] obtained similar results using the variegating *white* allele associated with translocation $T(1;3)w^{vco}$. Their methodology was seriously flawed, however, by constructing segmentally aneuploid histone gene deficiencies from translocation $T(Y;2)$ chromosomes[12]. In addition to deleting euchromatic portions of the chromosome, this technique produces poorly defined deficiencies and duplications of Y chromosome material. The Y chromosome contains regions which are potent suppressors of variegation[13] and whose integrity in $T(Y;2)$ chromosomes is questionable. We have tested a series of deficiencies, non-deficiencies and duplications of the histone gene cluster constructed from $(Y;2)$ translocations. The translocated Y chromosomes vary widely and unpredictably in their ability to suppress or enhance variegation. Hence, we have confined our study to the use of deficiencies restricted to small euchromatic portions of chromosome 2. These deficiencies allow us to control for any effect of Y heterochromatin by utilising uniform Y chromosomes. Of the five deficiency chromosomes tested, four (*Df(2L)1*, *12*, *65* and *84*) were generated from a chromosome bearing *Tft l(2)74i* while *Df(2L)161* was imposed on a *cn bw* chromosome. Although it is possible that the two source chromosomes vary with respect to undefined variegation modifying loci, this seems unlikely given the similarity of modification observed with the *Df(2L)65* and *Df(2L)161* chromosomes.

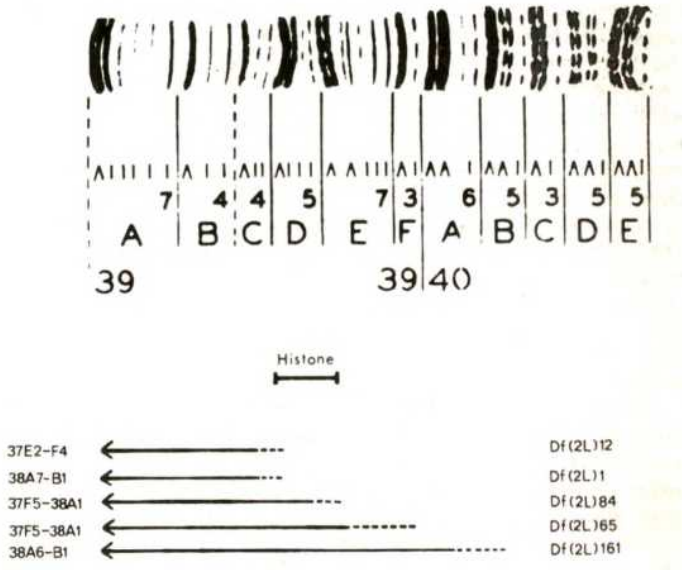


Fig. 1 A segment of the polytene chromosome map of *D. melanogaster* second chromosome showing the location of the histone gene cluster. The extent of each deficiency is indicated by a horizontal bar. Left breakpoints are listed; the right breakpoints fall within the broken portion of the bars. Cytological examination was originally performed by Wright *et al.*[6] and has been reconfirmed in our laboratory.

Our results do not entirely eliminate the possibility t
observed suppression of position–effect variegation is
deletion of a gene tightly linked to the histone
rather than deletion of the cluster itself. It sh
however, that heterozygotes for a control defic
left of the histone gene cluster (*Df(2L)1/+*
not exhibit more active expression of ei
than do the non-deficiency *CyO/+* si
Flies heterozygous for the deficiency
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results obtained, it must be tightly linked to the left of the cluster.

Reduction in the number of histone genes has not been directly correlated to a decrease in the amount of cellular histone protein; indeed, it seems improbable that the reduction in histone protein amount is equivalent to the reduction in gene multiplicity. However, we interpret our results to suggest that compensation for the reduced histone coding capacity is incomplete, and that the level of cellular histone protein is partially reduced during the period when the transcriptional fate of the variegating genes is determined, since reduction in the coding capacity for histone protein markedly affected the level of variegating gene expression.

Table 2 Mean percentage of wild-type (B^+) eye surface in $+/B^{SV}Y$ F_1 ♂ flies from the cross: $+/Y; CyO/Df(2L)$♂♂ × $\widehat{XX}/B^{SV}Y; +/+$♀♀

F_1♂genotype	No. of histone gene clusters	% B^+ eye size	S_Y	P
$+/B^{SV}Y; Df(2L)1/+$	2	53	1.9	<0.5
$+/B^{SV}Y; Df; CyO/+$	2	47	1.9	
$+/B^{SV}Y; Df(2L)12/+$	2	43	1.1	>0.05
$+/B^{SV}Y; CyO/+$	2	48	1.2	
$+/B^{SV}Y; Df(2L)84/+$	1–2	38	1.3	<0.05
$+/B^{SV}Y; CyO/+$	2	44	1.6	
$+/B^{SV}Y; Df(2L)65/+$	1	26	0.9	$\ll 0.05$
$+/B^{SV}Y; CyO/+$	2	46	1.1	
$+/B^{SV}Y; Df(2L)161/+$	1	18	0.7	$\ll 0.05$
$+/B^{SV}Y; CyO/+$	2	43	1.9	

F_1 progeny were reared at 21 °C. Approximately 100 eyes of each type were sketched with the aid of a dissecting microscope equipped with an ocular micrometer. Outlines were cut out and weighed to estimate relative surface area. An ANOVA test revealed no significant difference between $CyO/+$ sibling values. $Df(2L)/+$ values were compared to the sibling $CyO/+$ values using a t-test.

Intensity of gene inactivation is proportional to the fraction of salivary gland chromosomes in which the variegating gene locus has been heterochromatinised[14]. Our results implicate histone proteins as agents of heterochromatinisation. The role of Y chromosome heterochromatin in suppressing position–effect variegation can be envisaged as that of a competitor for free histone protein, which would otherwise be available to condense variegating gene loci[2]. Substances which affect the function of histone proteins in chromatin, such as sodium butyrate which inhibits histone deacetylation[15], can also be expected to have an effect on variegating gene expression. Modification of position–effect variegation may be useful property for testing presumptive mutations of histone gene function and multiplicity.

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Cis-active control of mouse β-galactosidase biosynthesis by a systemic regulatory locus

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Several higher organisms have been reported in which enzyme levels are determined genetically by sites located in close proximity to the corresponding structural genes. In several cases, these sites have been shown to act by controlling the rates of enzyme synthesis[1–4]. *Cis* compared with *trans* action has been tested for those proximate regulatory sites controlling enzymes for which appropriate structural variants exist[2,5–8]. The rate of synthesis of β-galactosidase in mouse tissues is under the control of a regulatory locus, *Bgl-s*, that is tightly linked to the enzyme structural gene[4,9]; we have tested the *cis*/*trans* nature of *Bgl-s* action by analysis of the electrophoretic mobility of the enzyme from animals heterozygous for the appropriate regulatory and structural alleles. Our results indicate that *Bgl-s* acts *cis*, controlling the expression of the structural gene located on the same chromosome.

A genetic complex, termed [*Bgl*], mapping near the *Mod*-1 locus on chromosome 9, contains within it elements governing the structure, development and rate of synthesis of β-galactosidase[4,9–15]. The structural gene *Bgl-e* is defined by an electrophoretic polymorphism which affects the enzyme in all tissues[9].

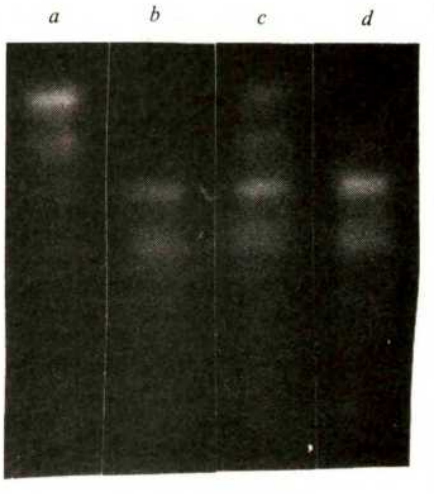


Fig. 1 Mobility patterns for kidney β-galactosidase as observed by enzyme activity stain. Kidneys from the appropriate animals were homogenised in 10 volumes (w/v) of 0.02 M imidazole-Cl, *p*H 7.4, containing 0.25 M sucrose. Homogenates were treated at *p*H 5.0 and 37 °C, and adjusted to a β-galactosidase concentration of 2 U ml^{-1}. Samples were subjected to isoelectric focusing on Ampholine PAG plates (*p*H 3.5–9.5) (LKB) for 2 h at 15 °C; β-galactosidase was stained by incubating the gels for 30 min in 0.1 M sodium citrate, *p*H 4.1, containing 0.05% Triton X-100 and 0.25 mM 4-methylumbelliferyl-β-D-galactoside (Sigma). Bands were observed and photographed under long wavelength ultraviolet light. *a*, Strain C57BL/6J, genotype $Bgl\text{-}e^{b/b}s^{h/h}$; *b*, strain B10.C(47N)/Sn, genotype $Bgl\text{-}e^{a/b}s^{h/h}$; *c*, F_1 (C57BL/6J × B10.C(47N)/Sn), genotype $Bgl\text{-}e^{a/b}s^{h/h}$; *d*, F_1 (B10.C(47N)/Sn × B6.CBA-$Trf^aBgl\text{-}s^d$), genotype $Bgl\text{-}e^{a/b}s^{h/d}$. B10.C(47N)/Sn animals were obtained from Dr M. Cherry, Jackson Laboratory; B6.CBA-$Trf^aBg\text{-}s^d$ animals were obtained from Dr Verne Chapman, Roswell Park Memorial Institute; C57BL/6J animals were purchased from the Jackson Laboratory.

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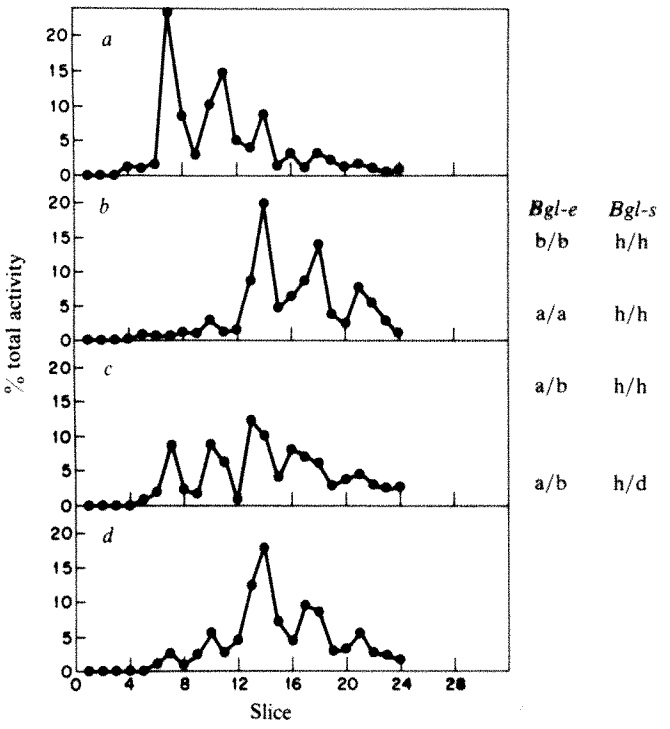


Fig. 2 Mobility patterns for kidney β-galactosidase as observed by direct assay. Kidney homogenates were prepared and isoelectric focusing was performed as described in Fig. 1 legend. After electrophoresis, gels were cut by hand into 2-mm slices, each slice was incubated for 2–4 h in 0.8 ml of 0.1 M sodium citrate containing 0.05% Triton X-100 and 0.25 mM 4-methylumbelliferyl-β-D-galactoside at 37 °C. After addition of 0.6 ml of 1 M sodium carbonate, fluorescence was measured in an Aminco fluorimeter[9]. The percentage of total activity recovered was plotted as a function of the number of slices from the cathode. (*a*), (*b*), (*c*) and (*d*) are the same as in Fig. 1 legend.

The allele coding for the more acidic form is designated *Bgl-e*^{a} and that for the more basic form is designated *Bgl-e*^{b}. A systemic regulatory element, *Bgl-s*, controls β-galactosidase concentration in all tissues at all stages during development[10–12]. It is tightly linked to *Bgl-e* and controls the rate of β-galactosidase synthesis[4]. The high activity allele is designated *Bgl-s*^{h} while the low activity allele is designated *Bgl-s*^{d}. Finally, the developmental programme for β-galactosidase synthesis in liver is genetically controlled by a temporal element within [*Bgl*], termed *Bgl-t*, as well as by one or more elements separate from the complex[4,13,15]. This last aspect of β-galactosidase regulation will not be considered further in this report.

Analysis of the β-galactosidase phenotype in more than 100 inbred mouse strains has made possible identification of three haplotypes of the [*Bgl*] complex[13,15]. The [*Bgl*]^{B6} haplotype, exemplified by strain C57BL/6J, is *Bgl-e*^{b}*s*^{h}; the [*Bgl*]^C haplotype, exemplified by strain BALB/cJ, is *Bgl-e*^{a}*s*^{h}; finally, the [*Bgl*]^{D2} haplotype, exemplified by strain DBA/2J, is *Bgl-e*^{b}*s*^{d}. Table 1 summarises the biochemical and genetic relationships among the three haplotypes. Each haplotype is now available in the C57BL/6J or C57BL/10Sn genetic background, which seem to be equivalent with respect to effects on β-galactosidase expression[15]. The [*Bgl*]^C haplotype of strain BALB/cJ has been transferred genetically into the C57BL/10Sn background to produce congenic strain B10.C(47N)/Sn; similarly the [*Bgl*]^{D2} haplotype of strain CBA/J has been transferred genetically into the C57BL/6J background to produce congenic strain B6.CBA-*Trf*^{a}*Bgl-s*^{d}. The ability to construct animals heterozygous for the appropriate structural and regulatory elements of the [*Bgl*] complex within a constant genetic background allows a test of the *cis* compared with *trans* nature of *Bgl-s* action.

The mobility patterns after isoelectric focusing of kidney β-galactosidase from various animals were determined. Figure 1*a* shows the pattern for animals of strain C57BL/6J, which is homozygous for the [*Bgl*]^{B6} haplotype. The multiple banding pattern represents charge heterogeneity resulting from unknown post-translation events affecting the product of a single structural gene[14,16]. The same pattern is seen in C57BL/10Sn animals, which also carry the [*Bgl*]^{B6} haplotype, and in strains carrying the [*Bgl*]^{D2} haplotype, containing the *Bgl-e*^{b} structural allele. Figure 1*b* shows the mobility pattern for the enzyme from congenic strain B10.C(47N)/Sn, which is homozygous for the [*Bgl*]^C haplotype containing the structural gene coding for the more acidic enzyme. Figure 1*c* shows the enzyme pattern from F_1 progeny of a mating of strains C57BL/6J and B10.C(47N)/Sn; these animals are heterozygous for the [*Bgl*] complex in that they carry the [*Bgl*]^{B6} haplotype on one chromosome and the [*Bgl*]^C haplotype on the other. Because these haplotypes differ only at *Bgl-e* and not at *Bgl-s* (Table 1), the pattern represents equal expression of both structural alleles. Figure 1*d* shows the pattern in F_1 progeny arising from a mating of strains B10.C(47N)/Sn and B6.CBA-*Trf*^{a}*Bgl-s*^{d}; these animals carry the [*Bgl*]^C haplotype on one chromosome and the [*Bgl*]^{D2} haplotype on the other and are therefore heterozygous for both the *Bgl-e* structural and *Bgl-s* regulatory elements of the complex (Table 1). They show predominant expression of the *Bgl-e*^{a} structural type, which is *cis* to the high activity *Bgl-s*^{h} regulatory allele within the complex. Because the relative expression of the two structural alleles in the heterozygote parallels the relative levels in the respective parental strains, the *Bgl-s* genetic element governing these levels acts *cis*.

This conclusion is supported by slicing the gels and assaying the β-galactosidase activity in each slice. Figure 2 shows the results for the kidney enzyme from animals homozygous for the [*Bgl*]^{B6} haplotype (Fig. 2*a*), animals homozygous for the [*Bgl*]^C haplotype (Fig. 2*b*), F_1 heterozygotes carrying both the [*Bgl*]^{B6} and [*Bgl*]^C haplotypes (Fig. 2*c*), and F_1 heterozygotes carrying both the [*Bgl*]^C and [*Bgl*]^{D2} haplotypes (Fig. 2*d*). In the last case, the shift in mobility toward that of the *Bgl-e*^{a} structural type, which is *cis* to the high activity *Bgl-s*^{h} regulatory allele, supports the contention that the *Bgl-s* locus is *cis*-active.

The assignment of the *Bgl-s* element as *cis*-active is qualitative. Quantification of expression of each *Bgl-e* allele in F_1 heterozygotes is complicated by the charge heterogeneity of each allelic product. This heterogeneity, along with the dimeric nature of β-galactosidase in the conditions of these experiments[14], results in a complicated array of overlapping charge forms in *Bgl-e*$^{a/b}$ heterozygotes. This makes accurate determination of the level of expression of each *Bgl-e* allele impossible. Attempts to remove the charge heterogeneity by chemical

Table 1 [*Bgl*] haplotypes and their alleles at the *Bgl-e* and *Bgl-s* sites

[*Bgl*] haplotype	Alleles* *Bgl-e*	*Bgl-s*	Strains	Kidney β-galactosidase activity†	Relative rate of kidney β-galactosidase synthesis‡
B6	b/b	h/h	C57BL/6J	32.5 ± 5.8	1.33×10^{-4}
			C57BL/10Sn	34.9 ± 5.6	—
C	a/a	h/h	BALB/cJ	28.8 ± 4.9	1.31×10^{-4}
			B10.C(47N)/Sn	32.3 ± 4.2	1.50×10^{-4}
D2	b/b	d/d	DBA/2J	13.4 ± 1.9	0.59×10^{-4}
			CBA/J	12.6 ± 1.4	—
			B6.CBA-*Trf*^{a} *Bgl-s*^{d}	12.6 ± 1.2	0.51 ×

* The alleles of *Bgl-e* (determined by measuring β-galactosidase el… mobility in polyacrylamide disc gels or on isoelectric focusing … *Bgl-s* (determined by measuring enzyme activity in brain o… various strains have been listed previously[9].

† Activities are expressed as μmol of product form… weight of tissue ±s.d., using the fluorescent substra… galactoside, as previously described[9].

‡ Rates of synthesis were determined as prev… as the ratio of c.p.m. incorporated into β-ga… total protein after brief labelling with ^{3}H…

treatment (sulphydryl reagents, EDTA, periodate) or enzymatic treatment (neuraminidase, alkaline phosphatase, endoglycosidases) have been unsuccessful (ref. 14; F.G.B., unpublished work). Thus, quantitative measurement of expression of each structural allele must await more elaborate physicochemical techniques.

The lack of information concerning genetic fine structure of the [*Bgl*] region of chromosome 9 makes it difficult to speculate in detail on the mechanism of action of the *Bgl-s* element in regulating the rate of β-galactosidase synthesis. Our finding that *Bgl-s* acts *cis* is consistent with previous hypotheses that *Bgl-s* represents either a germ line change in the number of active β-galactosidase structural genes[11,12], or an alteration in a regulatory element adjacent to the structural gene[11]. Although *Bgl-s* and the structural gene are inseparable by recombination[9], the discordant distribution of the two determinants among inbred strains, as exemplified by the difference between the $[Bgl]^{B6}$ and $[Bgl]^{C}$ haplotypes, suggests that they are not identical sites. However, it has yet to be determined whether the *Bgl-s* site is located within the structural gene or is a closely linked element outside of it.

In summary, the *Bgl-s* locus, which determines the rate of β-galactosidase synthesis and is tightly linked to the structural gene within the [*Bgl*] complex on chromosome 9, seems to act *cis*, regulating the expression of the structural allele located on the same chromosome; it is therefore unlikely that it codes for a diffusible regulatory molecule. Other *cis*-active elements, mapping in close proximity to their respective structural genes, have been identified in higher organisms[2,5–8]. One of these, the *Gus-r* locus controlling androgen induction of β-glucuronidase in mouse kidney, has been shown to act at the level of enzyme synthesis[2]. The *Bgl-s* locus is interesting in that it is the first systemic regulator of enzyme synthesis for which *cis/trans* action has been testable.

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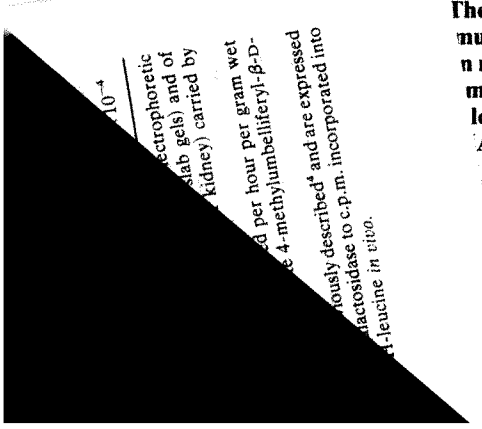
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Polymorphisms of human γ-globin genes in Mediterranean populations

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During normal human fetal development, two γ-globin genes are expressed to give γ-globin chains, one of which contains an alanine residue and the other a glycine residue at amino acid position 136 ($^{A}\gamma$ and $^{G}\gamma$, respectively)[1]. Recently, a human γ-globin chain mutant has been described in $HbF_{Sardinia}$ in which threonine replaces isoleucine at position 75 (ref. 2). This haemoglobin is frequently found in varying levels in Italian patients with β-thalassaemia (29 out of 32 patients), and in normal fetal cord blood in some populations (9 out of 19)[3]. In England the proportion of infants expressing this variant (usually at low levels but [illegible] **to 40% of the total fetal globin). The number of γ-globin** [illegible] **The two chains $^{A}\gamma$ and $^{G}\gamma$** [illegible] **mum possible number of** [illegible] **n ratio from 0.3 to 1.5 as** [illegible] **man and Schroeder to** [illegible] **loid genome[4,5]. Direct** [illegible] **A sequences gave data** [illegible] **Because of its wide-** [illegible] **d that the γ-chain in** [illegible] **gene, $^{T}\gamma$, non-allelic** [illegible] **by direct restriction** [illegible] **expressing $HbF_{Sardinia}$** [illegible] **nd therefore that the** [illegible] **e of either the $^{A}\gamma$- or**



Using the techniques of restriction enzyme analysis, we have recently studied the number and arrangement of γ-globin genes in humans. The map obtained is shown in Fig. 1. There are two γ-globin genes per haploid genome, separated by 3.5 kilobases. Each gene is split into at least two segments by an intervening sequence (intron)[10] at some position along the DNA coding for amino acids 100–121, the same position as for the human β- and δ-globin genes[3]. Other investigators[11,12] have also shown by molecular cloning that the $^{A}\gamma$-globin gene has two introns at precisely the same positions as those in the human β-globin gene. In our previous study[9], we had no information on the level of expression of $HbF_{Sardinia}$ in the individuals studied. Therefore, we still did not know whether the gene specifying the so-called $^{T}\gamma$ chain was a polymorphism of γ-globin gene number within the human population or whether the $^{T}\gamma$-globin gene was allelic

Table 1 Samples used in the study of γ-globin gene number in Mediterranean populations

Description	Origin	DNA sample source	Level of $HbF_{Sardinia}$
1. Normal	Dutch	Placenta	Unknown
2. Normal	British	Placenta	Unknown
3. Normal	Dutch	Cord blood	0%
4. Normal	Dutch	Cord blood	20%
5. Lepore	Southern Italy	Spleen	0%
6. β^0 thalassaemia	Southern Italy	Spleen	0%
7. β^+ thalassaemia	Northern Italy	Spleen	8%
8. β^0 thalassaemia	Sardinia	Spleen	20%
9. β^0 thalassaemia	Italy	Spleen	20%
10. β^+ thalassaemia	Southern Italy	Spleen	25%
11. β^0 thalassaemia	Northern Italy	Spleen	30%

The proportion of $HbF_{Sardinia}$ in cord blood samples of normal humans and peripheral blood samples of thalassaemia patients was estimated by separation of γ chains by CM-cellulose chromatography and subsequent tryptic digestion as detailed in ref. 17.

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with either the $^{A}\gamma$- or $^{G}\gamma$-globin gene, the two genes defined on the map we obtained[9]. The techniques of restriction enzyme site mapping[13] allow a simple test for this possibility.

We have made DNA from the spleens of seven β-thalassaemics and two non-thalassaemics with known levels of $HbF_{Sardinia}$ in their peripheral blood. The various samples used are detailed in Table 1. We have analysed the γ-globin gene region of these DNA samples. Briefly, this analysis involves digestion of the DNA with restriction endonucleases, denaturation and separation of DNA fragments according to size by electrophoresis on 1.0% or 1.2% agarose gels, transfer of DNA from gel to nitrocellulose filter sheets by 'blotting' according to the method of Southern[13], and detection of the fragments containing γ-globin gene sequences by hybridisation with ^{32}P-labelled pHγGl and subsequent autoradiography. pHγGl is a pCRI recombinant plasmid which contains a fragment of 500 base pairs of double-stranded $^{G}\gamma$-globin cDNA prepared from mRNA γ (ref. 14).

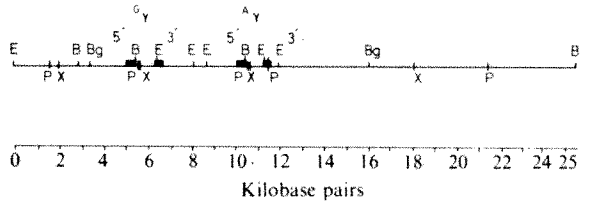


Fig. 1 The physical map of the human fetal globin genes. The position of each gene is marked by a filled box. Note that only those restriction enzyme sites that generate fragments containing part or all of the γ-globin genes are shown on this map. Although the genes are shown as split by a single intron, it is not possible to exclude the existence of further, small, introns in the $^{G}\gamma$-globin gene. The small intron in the $^{A}\gamma$-globin gene is not shown. The map is derived from ref. 9. E, *Eco*RI; B, *Bam*HI; Bg, *Bgl*II; P, *Pst*I; X, *Xba*I.

We have examined the γ-globin fragment patterns generated by *Eco*RI for all the samples in Table 1, and have also used the enzymes *Bgl*II, *Bam*HI (samples 3, 4, Table 1) and *Kpn*I (samples 5–11, Table 1). In all cases the size and number of fragments are identical to those given in Table 2, generated from normal individuals (samples 1, 2) shown previously to contain only two γ-globin genes per haploid genome[9]. These data strongly suggest that there are only two γ-globin genes per haploid genome present in individuals expressing $HbF_{Sardinia}$. An alternative explanation would be that a third γ-globin gene exists coding for the '$^{T}\gamma$' chain, but that in each digest it is present on a DNA fragment which co-migrates with one of the $^{A}\gamma$- or $^{G}\gamma$-globin gene fragments. We consider this unlikely; the fragment pattern is the same for DNA digested with four enzymes, whether they give separate $^{A}\gamma$ and $^{G}\gamma$ pieces (*Eco*RI and *Bam*HI), a fragment bridging the $^{G}\gamma$- and $^{A}\gamma$-globin genes (*Bam*HI), a fragment containing both genes (*Bgl*II) or a fragment containing the entire $^{G}\gamma^{A}\gamma\delta\beta$ complex (*Kpn*I, see ref. 15). The only reasonable objection to this would be a reduplication of the $^{G}\gamma^{A}\gamma\delta\beta$ locus; this is inconsistent with the available evidence on the inheritence of β-globin variants.

It could be argued that a putative $^{T}\gamma$-globin gene would not cross-hybridise well with the $^{G}\gamma$ cDNA plasmid probe used here due to extensive third position codon changes in the coding sequences. It would then be possible to miss new $^{T}\gamma$-globin gene-specific fragments because they would not be stable in the washing conditions used. To eliminate this possibility, *Eco*RI digests of three DNA samples from β-thalassaemic patients with 0%, 8% and 20% $HbF_{Sardinia}$, and DNA from a non-thalassaemic control known to contain only two γ-globin genes were hybridised with pHγGl and the filters subjected to increasingly stringent salt washes ranging from 3 × SSC (SSC = 0.15 M NaCl, 0.015 M Na citrate) to 0.01 × SSC at 65 °C (Fig. 2). Washing of filters at low salt concentrations (in this case, 0.1 × SSC) causes mismatched hybrids to melt, and as a result only hybrids between γ-globin gene sequences (both $^{A}\gamma$ and $^{G}\gamma$) and the γ cDNA plasmid probe are seen. Filters washed only with high salt (greater than 0.1 × SSC) show heterologous hybridisation (for example, with the β-globin gene sequences) in addition to the $^{A}\gamma$- and $^{G}\gamma$-globin gene hybridisation. In all conditions, the γ-globin gene-specific fragments seen are identical. This demonstrates that no third globin gene distantly related to that coding for $^{G}\gamma$-globin exists in persons expressing $HbF_{Sardinia}$ which would be absent in those people not expressing this protein.

The gene coding for the γ-globin chain of $HbF_{Sardinia}$ must, therefore, be allelic with either the $^{A}\gamma$- or the $^{G}\gamma$-globin gene. There is evidence[16,17] that the threonine residue at position 75 is present only in γ-globin chains that contain alanine at position 136 and we therefore conclude that the γ-globin chain of $HbF_{Sardinia}$ is an allele of the $^{A}\gamma$ gene.

The high level of occurrence of $HbF_{Sardinia}$ remains to be explained. In one study[3], the incidence of $HbF_{Sardinia}$ in β-thalassaemics in Italy was 90%, compared with only 40% in normal Italians. It is possible that the $HbF_{Sardinia}$ mutation is in linkage disequilibrium with either the β-thalassaemic gene or some other gene. It is not known whether the Ile 75 → Thr 75 substitution in $HbF_{Sardinia}$ alters the physiological properties of the haemoglobin molecule. It is therefore impossible to distinguish between mechanisms of selection, drift or linkage disequilibrium in accounting for the high frequency of this haemoglobin molecule. Indeed, the presence of the fixed mutations in $^{A}\gamma$ and $^{G}\gamma$ in the otherwise identical γ-globin chains seems to be an earlier example of a similar phenomenon.

As duplicated γ-globin genes have been demonstrated in the pig-tailed macaque (*Macaca nemestrina*)[18], orang-utan, gorilla and chimpanzee[19,20], we conclude that the duplication occurred at least 30 Myr ago[21]. It is remarkable that the two γ-globin genes have remained so similar during this time; some mechanism may exist to maintain sequence homology between these two genes.

Unequal cross-over events have been invoked to explain the conservation of multi-copy genes such as those for ribosomal RNAs[22–24]. This involves a contraction/expansion of gene numbers by unequal cross-over events and re-amplification by a further cross-over between two gene families of different sizes. In contrast to the ribosomal RNA genes, the only simple way in which a single γ-globin gene may be amplified is by a cross-over with a chromosome containing three γ-globin genes. This makes unequal cross-over a less attractive mechanism for sequence conservation. In addition, data on 810 humans showed that none of them synthesised only $^{A}\gamma$- or only $^{G}\gamma$-globin chains[25]. These experiments were not designed to detect 'heterozygotes' in which one chromosome had duplicated γ genes and the other had only a single γ-globin gene. From these data we calculate that the frequency of chromosomes carrying only a single γ-globin gene is less than 0.06 ($P = 0.05$).

It would be interesting to examine a large number of $HbF_{Sardinia}$ γ-globin chains at position 136. If there is a mechanism for spreading mutational changes between the $^{A}\gamma$

Table 2 Sizes of DNA fragments containing γ-globin genes generated by four restriction endonucleases

Enzyme	Fragment sizes (kilobase pairs)
*Bam*HI	15, 5.0, 2.6
*Eco*RI	6.5, 2.5, 1.65, 0.65
*Bgl*II	13
*Kpn*I	46

The human DNA used has been shown by restriction endonuclease mapping to contain only two γ-globin genes per haploid genome[17]. The relative positions of each fragment are as given in Fig. 1 or ref. 15.

and $^{G}\gamma$ genes it would be predicted that γ^{75Thr} should be found associated with γ^{136Gly}. The frequency of this occurrence would be an indicator of the rate of exchange of genetic information between $^{A}\gamma$ and $^{G}\gamma$. A precise study of the $HbF_{Sardinia}$ mutation would provide an interesting insight into mechanisms of gene conservation.

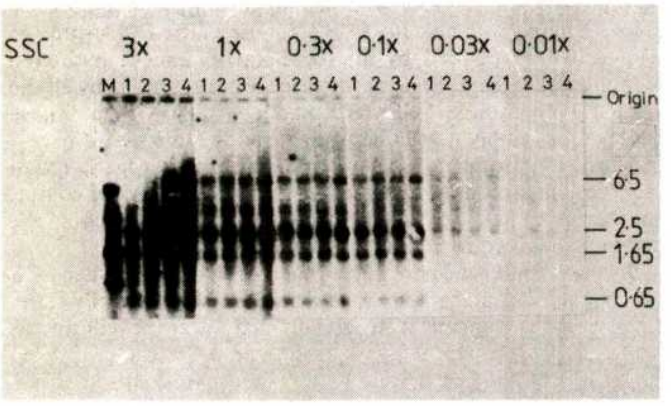


Fig. 2 The pattern of γ-gene fragments generated by *Eco*RI. DNA digestion, gel electrophoresis and hybridisation were carried out as in ref. 17. After hybridisation, duplicate filters were washed in various concentrations of SSC buffer (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, *p*H 7.5, 0.1% (w/v) SDS). The weakly hybridising bands at approximately 4–5 kilobases are due to cross-hybridisation between the γ-globin probe and β- and ε-globin gene sequences. Samples used expressed different levels of $HbF_{Sardinia}$: 1, 20%; 2, 8%; 3, 0%; 4, control DNA; and correspond, respectively, to samples 5, 7, 8 and 2 in Table 1. Sizes are in kilobases.

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Relationships between sister chromatid exchange and mutagenicity, toxicity and DNA damage

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Reciprocal exchanges of DNA in sister chromatids (SCEs) are induced by various carcinogens and mutagens[1–5], although the quantitative relationship between the number of mutations and SCEs induced varies among chemicals[5]. Nevertheless, the analysis of SCE production by various agents is often proposed as a sensitive and quantitative assay for genetic damage of the sort leading to mutation and cancer. In V-79 Chinese hamster cells we have been measuring DNA damage by alkaline elution, mutation induction as detected by 6-thioguanine resistance, and cytotoxicity as detected by colony formation for different physical and chemical agents. Some of the agents produced varying forms of DNA damage but undetectable increases in either mutation or toxicity. We report here that some undetectably mutagenic and/or toxic agents produce increases in SCE frequency and that DNA single-strand breaks, DNA–DNA interstrand cross-links, and DNA–protein cross-links are not necessary for SCE.

The toxic, mutagenic and DNA-damaging properties of the agents used here are summarised in columns (1)–(7) of Table 1. All the data in Table 1 were obtained from the same line of V-79-4 previously described[6,7]. Toxicity was measured by the loss of cell plating efficiency[6,7], mutation was measured as resistance to 6-thioguanine[6,7] over a complete dose range that inactivated up to 90% of the cells, and DNA single-strand breaks, DNA–protein cross-links and DNA–DNA cross-links were measured by alkaline elution[8–10]. Both *cis*- and *trans*-Pt(II) diammine dichloride (PDD) make DNA–DNA and DNA–protein cross-links, but the *cis* isomer is highly mutagenic in a dose-dependent manner whereas the *trans* isomer is not detectably mutagenic over a dose range of 25–450 μM (ref. 8). Both the fluorescent lamp and the sunlamp cause mutation and toxicity as well as DNA single-strand breaks and DNA cross-links[6,10,11]. If a filter of *p*-aminobenzoic acid is placed between the lamps and the cells, only light of wavelength >345 nm passes through to the cells. At the doses used, this light produced DNA single-strand breaks and DNA–protein cross-links but no detectable mutation or toxicity[10]. Hydrogen peroxide produces both toxicity and DNA single-strand breaks but is not detectably mutagenic even at doses up to 530 μM (ref. 12).

In these experiments the doses of the toxic agents were chosen to give approximately 10–45% survival (exact value given in Table 1). The SCE frequency was measured in V-79 cells by the technique described by Perry and Wolff[13]. Two flasks of the V-79 cells (5×10^5 cells per 25-cm^2 flasks in log growth) in each group were treated with the doses shown in Table 1. Immediately after treatment the medium was changed to one containing 10 μM bromodeoxyuridine. The cells were kept at 37 °C in the dark for two cycles of DNA replication before treatment with colcemid (0.1 μg ml^{-1}). Preliminary growth curves were first measured to determine the time after treatment for two population doublings to occur. The mitotic cells were collected by trypsinisation 2 h after the start of colcemid treatment. Drops of cells in fixative were placed on clean microscope slides and allowed to dry in air. The air-dried preparations were stained by the fluorochrome plus Giemsa procedure[13] and scored for SCE by one person who had no prior knowledge of the treatments. At least 60 metaphases were scored for each treatment group.

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The results of the SCE measurements are shown in columns (8) and (9) of Table 1. The statistical significance of the results was determined by the Wilcoxon rank sum test[14], a non-parametric method of analysis. *trans*-PDD, which is not detectably mutagenic, produces almost as many SCEs as does the highly mutagenic *cis*-PDD. As neither of these drugs produce DNA single-strand breaks, SCEs must be due to some other lesion, such as DNA–DNA and/or DNA–protein cross-links (Table 1). Although approximately equal numbers of SCEs are made by the two drugs at these concentrations, *trans*-PDD produces approximately 20-fold higher levels of DNA–protein cross-linking than does *cis*-PDD but equal numbers of DNA–DNA cross-links[8]. Therefore, in this instance, a closer quantitative relationship exists between DNA–DNA cross-links and SCEs than between DNA–protein cross-links and SCEs.

The radiation emitted from the sunlamp (General Electric 275-W sunlamp) produces many SCEs in V-79 cells (Table 1). The radiation was filtered with 3 cm of a 5-g l^{-1} solution of potassium hydrogen phthalate to allow only wavelengths >305 nm to pass through. The sunlamp has a broad emission spectrum in the near UV and visible regions, with peaks at the mercury emission lines[15]. In these conditions, the sunlamp causes mutagenicity, toxicity, DNA–protein cross-links and DNA single-strand breaks[10,11]. However, when a second filter of *p*-aminobenzoic acid is used to block wavelengths below 345 nm, the sunlamp radiation is no longer either mutagenic or toxic, but still producesce numbers of SCEs. Thus, SCEs can be formed in conditions that produce neither detectable toxicity nor mutagenicity.

The fluorescent lamp radiation induces small but significant ($P < 0.01$) increases in SCE frequency (Table 1). The *p*-aminobenzoic acid filter reduces the mutagenicity and toxicity of the light to undetectable levels whereas the number of SCEs is slightly decreased although still significantly higher than the controls ($P < 0.05$). Again, toxicity and mutagenicity are not correlatedhq SCE frequency.

Hydrogen peroxide produces toxicity and large numbers of DNA single-strand breaks but no detectable mutagenicigi[2]. Nevertheless, it causes significant increases in the SCE frequency, as seen in Table 1.

All the agents except *cis*- and *trans*-PDD make single-strand breaks and/or alkali-labile lesions in DNA. These platinum compounds produce the greatest increase in the frequency of SCEs, so single-strand DNA breaks are not required for SCE production. Hydrogen peroxide and both the filtered and unfiltered fluorescent lamp and sunlamp make SCEs but no detectable DNA–DNA cross-links, suggesting that these lesions are not uniquely involved in SCE formation. The DNA–protein cross-link is produced by all these agents except hydrogen peroxide. Therefore, none of the three lesions is made by all the agents, even though SCEs are made by all of them. These observations imply that none of the lesions examined is necessary for SCE production, although each of them, together with other lesions, could be sufficient for SCE formation. Of course, another lesion(s), not measured in these studies, could be uniquely involved in SCE formation.

The data presented here imply that correlations between SCE frequency and mutagenicity or toxicity are not necessarily close. Although it is difficult to be certain that an agent will not be weakly mutagenic in any conditions, nevertheless, given the limitations of the V-79 assay, four of the treatments in Table 1 are not detectably mutagenic but increase SCE frequencies. Resistance to 6-thioguanine as an assay for mutant colonies should score for many kinds of genetic or epi-genetic alterations in the enzyme hypoxanthine–guanine phosphoribosyl transferase. Although other genetic markers might give different quantitative results, we suggest that the qualitative conclusions would be similar.

The findings that *trans*-PDD and the filtered sunlamp cause large increases in SCE but no detectable mutagenicity suggest that the lesions causing SCEs in these cases differ from those causing 6-thioguanine-resistant mutations. Toxicity and SCEs are not necessarily correlated, as previously suggested by Wolff *et al.*[4], because the filtered sunlamp and fluorescent lamp both increase SCE frequency but are not detectably toxic.

Recent work by Kinsella and Radman[16] supports our contention that increased SCE frequency is not necessarily equivalent

Table 1 Relationships between sister chromatid exchanges, mutagenicity, toxicity and DNA damage

Agent	Quantity of agent	% survival (±s.e.m.)	Mutant frequency relative to control*	DNA Single-strand breaks and/or alkali-labile lesions†	DNA–Protein cross-links†	DNA–DNA cross-links†	SCEs per chromosome (±s.e.m.)	Significance compared with controls by Wilcoxon rank sum test	Refs for data in columns (3)–(7)
Control	–	100 ± 4.8	1	–	–	–	0.351 ± 0.23	–	–
trans-Pt (II) diammine dichloride	250 μM	16 ± 1.0	1	±‡	++	+	2.911 ± 0.131	$P < 0.01$	8
cis-Pt (II) diammine dichloride	10 μM	11 ± 0.5	40	–	+	+	3.960 ± 0.200	$P < 0.01$	8
GE 275-W sunlamp	2.4×10^5 Jm^{-2}	45 ± 3.6	23	++	+	–	1.772 ± 0.135	$P < 0.01$	10,11
Sunlamp plus *p*-amino benzoic acid filter	1.8×10^5 Jm^{-2}	100 ± 5.4	1	+	+	–	1.556 ± 0.139	$P < 0.01$	10,11
Sylvania F15T8 cool white fluorescent lamp	5.9×10^4 Jm^{-2}	35 ± 3.2	53	++	+	–	0.580 ± 0.034	$P < 0.01$	6,10
Fluorescent lamp plus *p*-aminobenzoic acid filter	$3.2. \times 10^4$ Jm^{-2}	100 ± 3.2	1	+	+	–	0.452 ± 0.019	$P < 0.05$	6,10
Hydrogen peroxide	353 μM	20 ± 1.6	1	++	–	–	0.616 ± 0.048	$P < 0.01$	12

Per cent survival was determined as the decrease in plating efficiency relative to the controls. The control plating efficiency varied between 75 and 95%.

* The numbers are the mutant frequency of the experimental group divided by the mutant frequency of the controls. Mutant frequency is defined as the number of colonies resistant to 6-thioguanine per survivor in appropriate expression and selection conditions as previously described[6,7]. 1 Indicates that the treatments were not detectably mutagenic as defined by the lack of a dose–response relationship. The mutant frequencies of the various controls were within the limits of $1.3 \pm 0.9 \times 10^{-6}$.

† The symbols are: –, no lesions detectable; ±, barely detectable lesions; +, small numbers of lesions; ++, large numbers of lesions.

‡ The quantitative significance of the small number of apparent breaks has not been determined.

to increased mutant frequency. These workers have shown that the non-mutagenic tumour promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induces SCEs, whereas the non-promoting derivative 4-*O*-methyl TPA does not. Further support comes from the finding that saccharin also induces SCEs in both hamster and human cells[17], although it is considered to be non-mutagenic[18,19]. From these and our own results, we conclude that, although the analysis of SCEs can often be very useful, definitive interpretations of SCE data await a molecular mechanism for their formation.

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Intranuclear injection of anti-actin antibodies into *Xenopus* oocytes blocks chromosome condensation

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The role of contractile proteins in the structural organisation of the interphase nucleus and of metaphase chromosomes is largely unknown. Actin has been found in interphase nuclei of different species, especially in association with condensed chromatin[1–6]. In the germinal vesicle (nucleus) of *Xenopus* oocytes, actin has been localised in the nuclear gel supporting the chromosomes and the extrachromosomal nucleoli[7–10]. It has been reported that the premeiotic lampbrush chromosomes in these germinal vesicles are positively stained for actin and tubulin by the immunoperoxidase technique[11]. Moreover, the longitudinal contraction of these chromosomes is ATP dependent[12]. Therefore it has been suggested that actin participates in the structural organisation of the highly specialised lampbrush chromosomes. However, actin is not a major component of the metaphase chromosome scaffold[13]. The results reported here suggest that actin is involved in the condensation of *Xenopus* chromosomes.

We studied chromosome condensation and meiotic spindle formation in the presence of antibodies directed against actin and myosin in maturing oocytes of *Xenopus laevis*. Affinity purified human anti-actin antibodies (AAA), characterised elsewhere[14,15], and a γ-globulin fraction from rabbit serum containing antibodies against human uterine myosin (AMA)[16] were injected into the cytoplasm or into the germinal vesicle of fully grown oocytes. As a control, we injected nonspecific human or rabbit γ-globulins. The injection technique has been described in detail elsewhere[17]. Progesterone was added to induce *in vitro* maturation[18]. The hormone treated oocytes undergo breakdown of the germinal vesicle and the first meiotic division, and then remain arrested in the second meiotic metaphase. The eggs were fixed, embedded in paraffin, cut

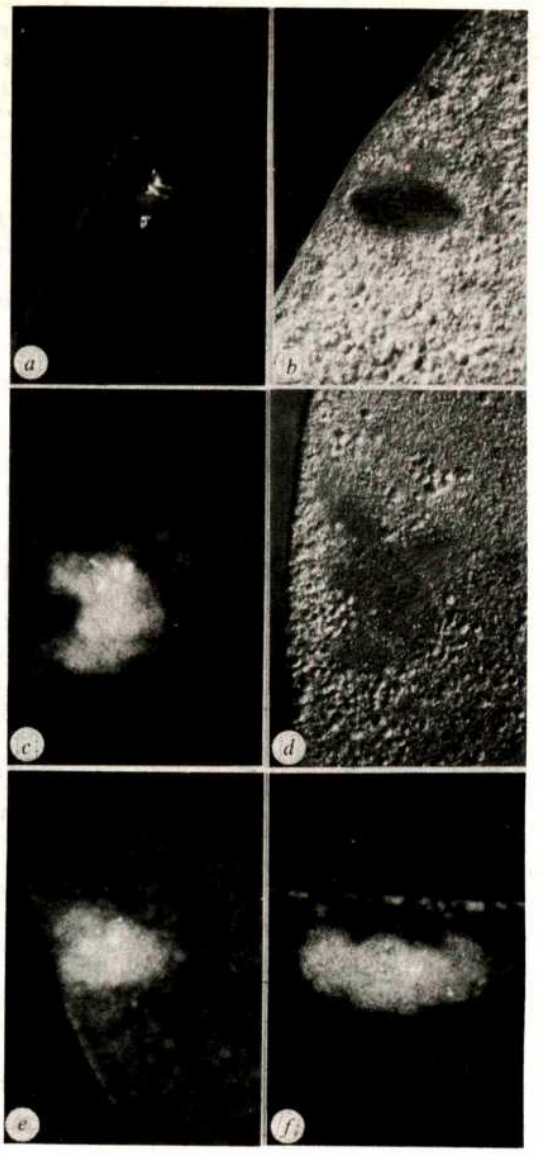


Fig. 1 Effect of AAA injection into the germinal vesicle of *Xenopus* oocyte on chromosome condensation and spindle formation. Fully grown *Xenopus laevis* oocytes were centrifuged[19] (400*g*, 10 min, allowing nuclei to sink back into the cytoplasm after injection) and injected intranuclearly with 20 nl of injection buffer containing 0.4 mg per ml of either AAA or nonspecific human γ-globulins (Fluka) equivalent to 8 ng of proteins per nucleus. After 1 h, 10^{-5} M progesterone was added to induce meiosis. Oocytes showing a distinct white spot indicative of maturation were fixed in Smith's solution[20], dehydrated in butanol, embedded in paraffin, cut into 6-μm thick serial sections, stained by the Feulgen technique and counterstained with light green. *a*, Fluorescence micrograph at wavelength 546 nm to demonstrate Feulgen-positive chromosomes in metaphase of an egg injected with nonspecific γ-globulins. Condensed chromosomes are normally organised on the spindle. *b*, Nomarski differential interference microscopy of the same field; the spindle appears normal. *c*, Fluorescence micrograph of Feulgen-positive material in a meiotic metaphase of an egg injected with AAA. Chromatin appears as a loosely arranged large spot with dense bodies scattered between. Note the positivity in the areas corresponding to asters. *d*, Nomarski differential intereference microscopy of the same field as in (*c*); asters are present but the spindle is disorganised. *e*, And *f*, fluorescence microscopy of other examples of loose chromatin in meiotic metaphases of eggs after injection of AAA (×700).

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serially and stained by the Feulgen technique. Our experimental system, which thus facilitates cytological observation of chromosome condensation and spindle formation, is described in detail in the legend to Fig. 1.

Centrifuged oocytes injected with nonspecific human or rabbit γ globulins into either the cytoplasm or the nucleus produced normal meiotic spindles in three series of experiments. After nuclear injection of nonspecific γ-globulins, seven normal spindles were found in eight cases (Fig. 1*a* and *b*), the remaining egg having probably lost its spindle and chromatin through the injection hole—no Feulgen-positive material was found on any of the serial sections. In a few cases, we observed anaphase or telophase of the first meiotic division instead of second metaphase. This does not influence the observation of chromosome condensation because there is no despiralisation of chromosomes between the two meiotic divisions. These observations show that normal meiosis takes place in the oocytes after chemical defolliculation, centrifugation and nuclear injection of nonspecific human or rabbit γ globulins.

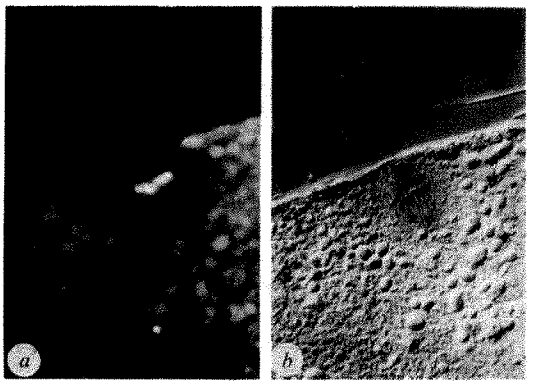


Fig. 2 Normal meiotic metaphase after injection of AMA into the nucleus of a *Xenopus* oocyte. Techniques are explained in the legend to Fig. 1. Nuclei were injected with 20 nl of injection buffer containing 2 mg per ml AMA γ-globulin fraction (DE 52 cellulose column) equivalent to 40 ng of proteins per nucleus. *a*, Fluorescence micrograph showing condensed Feulgen-positive chromosomes normally organised on the spindle. *b*, Nomarski differential interference microscopy of the same field; the spindle appears normal (×700).

Injection of AAA into the cytoplasm did not affect germinal vesicle breakdown, chromosome condensation or spindle formation, although three times more AAA was injected than into the nucleus. In all five cases, normally condensed chromosomes were observed, associated with normal spindles.

By constrast, the injection of AAA into the germinal vesicle of the oocyte severely affected chromosome condensation. In none of the 11 oocytes examined from three independent series were normal meiotic metaphase chromosomes formed. In all nine cases where chromatin was found in the sections, it was loosely arranged but in part associated with spindle asters. Figure 1*c–f* shows examples of the deficient chromosome condensation in the presence of intranuclear AAA. No effect on normal meiotic metaphase formation was observed after injection of rabbit γ-globulins containing AMA into the nucleus (Fig. 2).

These results show that in our experimental conditions, AAA, but not AMA, affected chromatin condensation when injected into the germinal vesicle of *Xenopus* oocytes. The lack of effect of AMA does not necessarily mean that myosin does not have a role in chromosome condensation. Alternatively, we could have used insufficient AMA, nuclear *Xenopus* myosin could be antigenically different from human uterine myosin, or the binding of AMA to myosin may not affect the activity of the protein in this respect. We do not know the mechanism of the inhibitory action of AAA on chromosome condensation. Since AAA are precipitating antibodies[15], it is possible that they precipitate out the amount of protein that is instrumental in chromosome condensation.

After intracytoplasmic injection of AAA, metaphase spindles looked normal, perhaps because the nuclear envelope acted as a barrier—chromosome condensation probably starts before germinal vesicle breakdown. Moreover, large quantities of cytoplasmic actin could bind to the injected AAA and block their activity.

Actin and myosin have been described as constituents of the mitotic spindle[21–25]. In our experiments, mitotic spindle formation was perturbed after intranuclear injection of AAA (Fig. 1*d*). However, asters were formed and small portions of the loose chromatin were found closely associated with the aster fibres (Fig. 1*c*). These experiments thus do not elucidate the possible role of actin in the attachment of chromosomes to spindle fibres and in spindle pole formation.

At any rate, our results suggest that actin is involved in chromosome condensation. Further work is needed to clarify whether actin exerts its influence by being an integral component of the prophase chromosome structure.

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Mouse epidermal Ia molecules have a bone marrow origin

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The major histocompatibility or H-2 complex of the mouse is divided into five regions (K, I, S, G and D)[1]. Genes in the I region regulate immune responses[2]. The I region has several subregions which are designated I-A, I-B, I-J, I-E and I-C. The I-A and I-E subregions also code for a set of serologically detected cell-surface alloantigens, designated Ia antigens[3]. The relationship between the genes regulating the immune

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response and those encoding the serologically detectable alloantigens is still unknown. A number of species including man, rats and guinea pigs contain genetic regions apparently equivalent to the murine I region. Ia molecules are integral cell-surface glycoproteins that consist of two subunits of approximate molecular weights 35,000 (α) and 28,000 (β). Unlike the classical transplantation antigens which are present on almost all cells, the Ia antigens are found primarily on cells of the immune system—lymphocytes and macrophages[4–6]. A notable exception has been the demonstration of Ia antigens in mice, or Ia-like antigens in other mammals, on epidermal cells[6–13]. There is controversy about the numbers of Ia-positive cells in the epidermis. Fluorescence studies in humans[11,12], guinea pigs[14] and mice[15] indicate that only about 5% of epidermal cells are Ia positive. These cells were identified by morphological criteria as the macrophage-like Langerhans cells. However, cytotoxicity studies in mice using anti-Ia sera indicate that a majority of epidermal cells (up to 90%) are Ia positive[6–8]. The reason for this discrepancy is not known. Here we demonstrate that the epidermal Ia molecules are synthesised by bone marrow-derived cells, presumably Langerhans cells.

We replaced the entire population of bone marrow-derived cells in one animal with bone marrow cells from a second animal capable of synthesising Ia molecules distinct from the first. These chimaeric animals are constructed by the injection of donor bone marrow cells into lethally irradiated recipients. In chimaeric animals all cells except those of bone marrow origin are of the recipient genotype. We reasoned that if the Ia molecules synthesised in epidermal cell preparations (mouse tail cells) are synthesised by bone marrow-derived cells, the Ia molecules isolated from long-term (>3 months) chimaeras should be of donor origin. Alternatively, if the Ia molecules are synthesised by a majority of epidermal cells, the Ia molecules should be of recipient origin.

Table 1 Source of haematopoietic cells in chimaeric mice

Target	Anti-K^b H-2.33*	Anti-D^b H-2.2†	Anti-K^k H-2.23*	Anti-D^d H-2.4†
Donor→Recipient				
IR→(B10×1R)F_1	<10 (<10)	NT	320 (>90)	NT
(B6×A)F_1→B6	NT	80	NT	>320
Controls				
1R	<10 (<10)	160	320 (>90)	<10
B10	>640 (>80)	160	<10 (<10)	<10
A	<10 (<10)	<10	320 (>90)	>320
(B6×A)F_1	NT	80	NT	>320
(B10×1R)F_1	>640 (>90)	NT	320 (>90)	NT

Chimaeras were produced as described by Von Boehmer[20]. Mice were irradiated with 925 rads from a linear accelerator and reconstituted with $1–2\times10^7$ anti-mouse brain ($\alpha\theta$) plus complement-treated bone marrow cells. Mice were tested for chimaerism after 3 months. NT, not tested.

*Tested by dye exclusion microcytotoxicity on peripheral blood leukocytes. Numbers represent reciprocal of the antiserum dilution giving 50% killing of targets and in parentheses, the maximum attainable killing. Sera for cytotoxicity: anti-H-2.33 (A×B10.D2)F_1 anti-B10.A(5R); anti-H-2.23, (B10×A.TL)F_1 anti-A.AL.

†Tested by polyvinylpyrrolidone (PVP) haemagglutination of red blood cells. Number represents the reciprocal of the greatest antiserum dilution which permits detectable agglutination reaction. Sera for haemagglutination: anti-H-2.2, (A×B10.D2)F_1 anti-B10.A(2R); anti-H-2.4, (B10.A(1R)×A.SW)F_1 anti-B10.A.

Our experimental design requires almost complete replacement of recipient bone marrow-derived cells with donor cells. Direct tests confirmed that this had occurred (Table 1). Two types of chimaeric animal were constructed: parental (P) cells were transplanted to F_1 offspring (P→F_1) and vice versa (F_1→P). For the P→F_1 chimaeras, the analysis of bone marrow-derived cells used cytotoxicity and haemagglutination assays. In the case of the F_1→P chimaeras, the donor cells in the recipient were measured by haemagglutination. In both cases, the results indicated that the haematopoietic system was almost completely reconstituted with donor cells (Table 1).

We isolated epidermal Ia molecules by indirect immunoprecipitation using mouse alloantisera as previously described[10]. The specificity of the antisera is critical in determining whether the Ia is contributed by donor or recipient cells in these chimaeras. The sera were characterised extensively by cytotoxicity, absorption and immunoprecipitation (data not shown). They were tested on strains identical to those used in these experiments to demonstrate unequivocally that the observed reaction was specific for recipient or donor strains. An F_1 animal

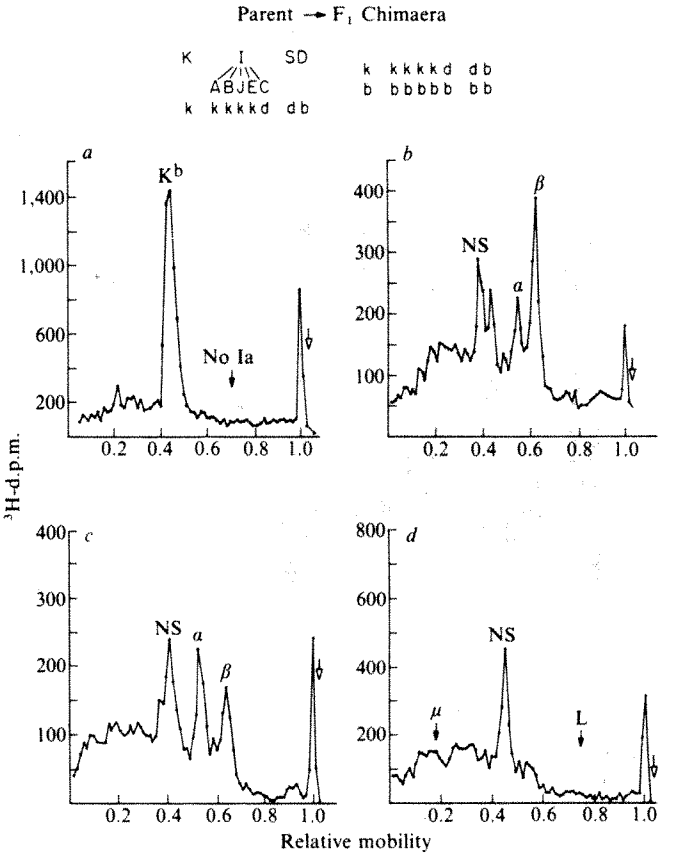


Fig. 1 SDS-polyacrylamide gel electrophoresis of epidermal Ia molecules isolated from parent→F_1 bone marrow chimaeras. Epidermal Ia was isolated as previously described[10]. Briefly, single cell suspensions of trypsinised epidermal cells were biosynthetically labelled with ^{3}H-amino acids and lysed in 0.5% NP40. The glycoprotein fraction was isolated by *Lens culinaris* lectin affinity chromatography, concentrated fivefold in a B-15 Minicon, and indirectly immunoprecipitated with specific antisera and fixed *Staphylococcus aureus* (Cowan I strain). The parent→F_1 chimaera extracts were precipitated with (A×B10.D2)F_1 α B10.A(5R) [$\alpha K^b I–A^b I–B^b$] (*a*) (B10×HTI)F_1 α B10.A(5R) [$\alpha I–E^d I–C^k$] (*b*) (A.TH×B10.HTT)F_1 α A.TL[$\alpha I–A^k I–B^k I–J^k$] (*c*) rabbit anti-MOPC 104E [R$\alpha\mu$] (*d*). ↓Arrows indicate MOPC 104E heavy chain (μ) and light chain markers; | arrows indicate the dye front. NS represents nonspecific 45K peaks, possibly actin.

[B10.A(1R)×B10] is heterozygous for the H-2 complex and expresses Ia molecules encoded by both the H-2^{h1} and H-2^b haplotypes (Fig. 1). In F_1 chimaeras (P→F_1), only Ia molecules from the donor animal can be isolated from epidermal cells. Both I-A and I-E subregion products can be detected (Fig. 1*b*, *c*). In these chimaeras, we cannot detect Ia molecules of the H-2^b haplotype from the B10 parent (Fig. 1*a*). In contrast, transplantation antigens of both haplotypes can readily be detected in these chimaeric animals. The results in Fig. 1*a* also provide direct evidence that transplantation molecules (K^b) can be synthesised by a non-bone marrow-derived cell. We should stress that immunoprecipitation with a rabbit anti-μ serum

showed no evidence of contaminating IgM-positive cells in the epidermal cell population (Fig. 1*d*). Thus, the donor Ia must be derived from a population of bone marrow cells distinct from B cells.

In the chimaera described above, we could only demonstrate a loss of one haplotype of Ia antigens from tail cell preparations derived from P→F_1 chimaeras. To demonstrate directly the appearance of new Ia antigens not present in the host, F_1→P chimaeras were constructed. The [B6×A]F_1 animal has Ia molecules of the H-2^b (B6) and H-2^a (A) haplotypes. In [B6×A]F_1→B6 chimaeras, we could detect the presence of Ia molecules of both the H-2^a and H-2^b haplotypes by immunoprecipitation. I-A Subregion products from both H-2^b and H-2^a haplotypes were easily detected (Fig. 2*a*, *b*). The A^k molecules can only be derived from the bone marrow cells. Both A^k and E^k molecules are present in these chimaeras (E^k not shown). Thus, new epidermal Ia antigens are derived from the donor bone marrow cells. These data from the P→F_1 and F_1→P chimaeras strongly suggest that the Ia molecules from chimaeric epidermal cells originate from bone marrow-derived cells.

The original reports of Ia molecules on mouse epidermal cells were based on adsorption analysis and antibody-mediated cytotoxicity using dye exclusion[6]. Other investigators independently confirmed these results[7,8]. Using an indirect immunofluorescence assay, investigators working with guinea pigs, man and mouse, have suggested that the Ia is confined to a small subpopulation of epidermal cells, the macrophage-like Langerhans cells[11,12,14,15]. In guinea pigs, biosynthetically labelled Ia molecules can only be immunoprecipitated from a Langerhans cell-enriched fraction, and not from a Langerhans cell-depleted fraction of epidermal cells[16]. Our experiments suggest that the biosynthetically labelled Ia molecules are derived from a cell of bone marrow origin. These observations suggest that a small number of cells in the epidermis are derived from the bone marrow and express Ia molecules. Although we have no direct evidence for the bone marrow origin of Langerhans cells, the fluorescence studies in guinea pigs, man and mouse[15], together with the present results, suggest that the Langerhans cell produces the epidermal Ia molecules and has a bone marrow origin. This possibility is not surprising considering the macrophage-like functions ascribed to the Langerhans cells[17,18]. Macrophages are generally thought to be derived from bone marrow precursors. However, our experiments detect only newly synthesised Ia molecules. The sensitivity of detection is a function of both the number of Ia molecules and their turnover rate. We are aware that Ia molecules could be synthesised by non-bone marrow-derived cells, and not detected in our experiments, if the Ia molecules were in much lower concentration per cell or turning over more slowly.

Differential sensitivity of immunofluorescence and cytotoxicity assays, or bystander effects in the cytotoxic assays or contaminating Sk antibody might explain the conflicting cytotoxicity[6–8] and immunofluorescence[11,12,14,15] data. At present, we feel the simplest interpretation of our data is that the Ia molecules present in the epidermis are synthesised by bone marrow-derived cells, probably the Langerhans cells.

Phagocytic macrophage cells are involved in the presentation of antigen to T cells. The subsequent activation and proliferation of the T cells involves the Ia molecules on the antigen-presenting macrophage[19]. In guinea pigs, Langerhans cells can function both as stimulators in a mixed lymphocyte reaction and as antigen-presenting cells[16]. Perhaps Langerhans cells in the epidermis function like macrophages[18]. A high concentration of antigen-presenting cells in the skin is reasonable, as the skin is the first line of defence against pathogens. It was suggested that non-lymphoid Ia-positive epithelial tissues also might be immunologically relevant[13]. The precise immunological implications of Ia molecules on cells in the epidermis are unknown. However, the observations reported here are intriguing because they clearly raise the possibility that Ia molecules may be restricted to cells involved in the immune response.

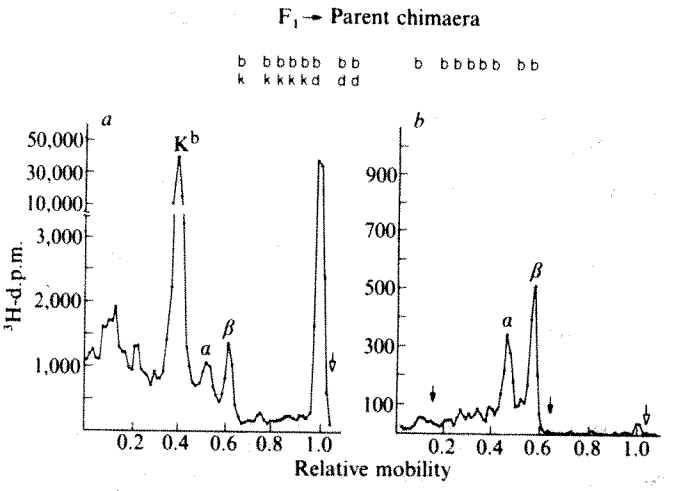


Fig. 2 SDS-polyacrylamide gel electrophoresis of epidermal Ia molecules isolated from F_1→parent bone marrow chimaeras. The F_1→parent chimaera extracts were precipitated with (A×B10.D2)F_1 αB10.A(5R) [$\alpha K^bI\text{-}A^bI\text{-}B^b$] (*a*) and CWBαC3H hybridoma 10-2.15 [$\alpha\text{-}A^k$] (*b*). This reagent has been characterised to react with A^k (ref. 21). We tested the hybridoma reagent using a two-stage dye exclusion cytotoxicity assay. No killing of B10 spleen cell targets was seen with this reagent (cytotoxic titre <1/10). A/Sn spleen cell targets were efficiently lysed (titre of >1/640 with 50% maximum cytotoxicity). In tail skin preparations from chimaeras, the ratio of transplantation antigens (K^b) to Ia molecules is altered compared with normal tail skin preparations[10] (*a*). In chimaeras, there are fewer Ia molecules relative to transplantation antigens. The reason for this altered ratio is not clear. It seems likely that it represents the partial repopulation of epidermal Ia-bearing cells from bone marrow precursors. The monoclonal antibody used in *b* always gives smaller nonspecific peaks than immunoprecipitations using alloantisera. See Fig. 1 legend for explanation of markers.

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Epidermal Langerhans cells are derived from cells originating in bone marrow

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Langerhans cells constitute a morphologically well characterised subpopulation (3–8%) of mammalian epidermal cells which, in contrast to the bulk of epidermal cells, bear Fc-IgG and C3 receptors[1], express immune response-associated (Ia) antigens and function as antigen-presenting cells and allogeneic stimulatory cells to primed T lymphocytes[2–5]. The ontogeny of Langerhans cells has been a subject of considerable debate since their discovery[6]. Although some studies suggest that Langerhans cells are of mesenchymal as opposed to neural or melanocytic origin[7], direct evidence for this has not been presented. In this study we demonstrate that, after 3 weeks, most of the Langerhans cells (LC) in parental skin which had been transplanted on to F_1 hybrids were of recipient origin whereas keratinocytes remained of donor origin; this indicates that the LC are derived from a mobile pool of cells. Furthermore, in studies of skin from radiation-induced bone marrow chimaeric animals we found that, depending on the strain combination, up to 80% of the epidermal LC were derived from the bone marrow of the donor animals.

Previous studies have demonstrated that in mice[8,9] as well as in guinea pigs and humans, H–2 (B region in guinea pigs and HLA-A or -B in humans) antigens are expressed on the great majority of epidermal cells. In contrast, Ia antigens or their equivalents in mice, guinea pigs and humans are detected only on epidermal Langerhans cells[1–5,8,9]. Langerhans cells can also be identified by light microscopy when they are incubated with IgG-coated ox red blood cells (EA-IgG), as they form rosettes through their Fc receptors. Our studies used epidermal cell suspensions prepared by trypsinisation (1% for 60 min at 37 °C), and epidermal cell-surface characteristics were identified by using a simultaneous rosette and indirect immunofluorescence (IF) assay[9].

First, we transplanted sex-matched parental skin from A/J mice on to CAF_1 (BALB/c×A/J) hybrid mice and, after various time periods, determined whether the H–2 and Ia antigens of the LC and other epidermal cells were those of the donor or recipient. Table 1 shows that using an anti-Ia.8 antiserum which detects the Ia of BALB/c but not of A/J mice, 3.5–4% of the epidermal cells of the A/J transplants bore Ia antigens characteristic of the CAF_1 mice. The great majority of these were LC, as from 61% (11 d post-transplantation) to 100% (49 d post-transplantation) of the Fc-IgG-rosetting cells (Langerhans cells) were positive with the anti-Ia.8 reagent. Using an anti-H–2^d antiserum [$(B10\times A)F_1$ anti-B10.D2] we found that most of the epidermal cells were not of CAF_1 origin. Furthermore when syngeneic transplants (A/J skin on to A/J mice) were carried out, the anti-Ia.8 antiserum did not stain any of the A/J epidermal cells whereas 4.2% of these epidermal

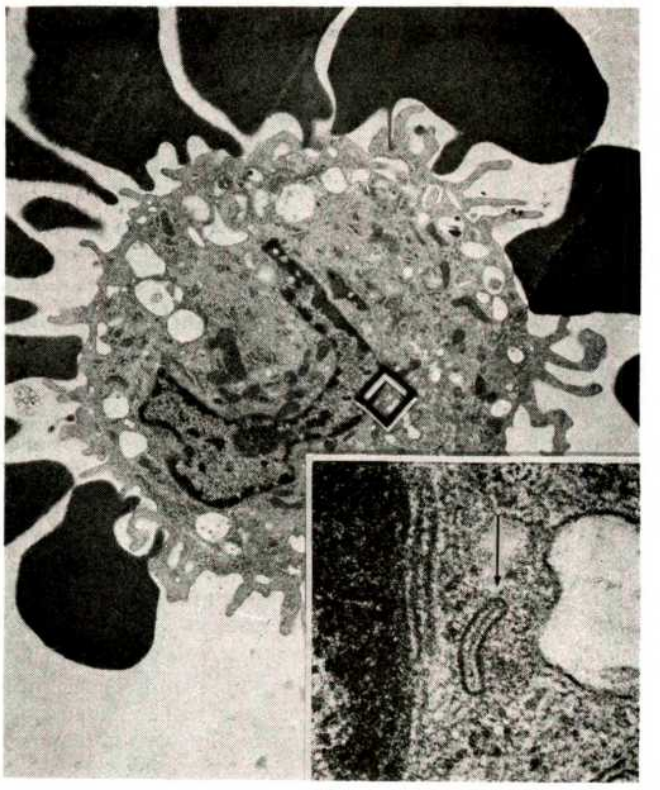


Fig. 1 Electron micrograph of an Fc-IgG-rosette-forming Langerhans cell which exhibits a lobulated nucleus, dendritic projections and typical Langerhans cell granule (×4,000, inset ×63,000).

Table 1 Detection of major histocompatability complex alloantigens on transplanted skin

Donor	Recipient	Day tested	Antisera: Serum 1 (anti-Ia^d)		Serum 2 (anti-Ia^d, H–2^d)		Serum 3 (anti-$Ia^{k,d}$)	
			%IF +ve*	%Rosette +ve†	%If +ve	%Rosette +ve	%IF +ve	%Rosette +ve
A/J	A/J	14	0	0	0	0	4.2	95
0	CAF_1 (A/J×BALB/c)		5	95	76	92	4.2	100
A/J	CAF_1	11	4‡	61	6.9	74	NT	NT
		14	3.5	61	8	60	NT	NT
		21	3.5	95	9	100	5	95
		49	3.8	100	8	100	5	90

The antisera used have been extensively characterised by cytoxicity and absorption analyses on spleen and lymph node cells. Serum 1 is a $(B10.A\times A)F_1$ anti-B10 which is negative on H–2^a(A/J) and when tested on H–2^d(BALB/c) reacts only with the I–A specificity Ia.8. Serum 2 is a $(B10\times A)F_1$ anti-B10.D2 which is also negative on H–2^a and when tested on BALB/c reacts with the H–2K specificities H–2.31 and the Ia specificities Ia.8 and 11. Serum 3 is an A.TH anti-A.TL which is a polyvalent anti-Ia antiserum, and reacts with multiple Ia specificities of both H–2^a and H–2^d. NT, Not tested.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two or three experiments.

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Table 2 Detection of major histocompatibility alloantigens on epidermal cells of chimaeric mice

Donor	Recipient	Day tested	Antisera: Serum 1 (anti-H-2^d, Ia^d)		Serum 2 (anti-H-2^b, Ia^b)		Serum 3 (anti-Ia^d)	
			%IF +ve*	%Rosette +ve†	%IF +ve	%Rosette +ve	%IF +ve	%Rosette +ve
BALB/c	C57BL/6	48	2.7‡	20	68	88	NT	NT
		62	2.9	45	70	58	NT	NT
		89	1.5	30	81	83	2.4	27
Controls								
BALB/c(H-2^d, Ia^d)			83	91	0	0	2.9	80
C57BL/6(H-2^b, Ia^b)			0	0	78	97	0	0

The antisera used have been extensively characterised by cytotoxicity and absorption analysis on spleen and lymph node cells. Serum 1 is a $(B10 \times A)F_1$ anti-B10.D2 which is negative on H-2^b and when tested with BALB/c reacts with the H-2K specificities H-2.31 and the Ia specificities Ia.8 and 11. Serum 2 is a B10.D2 anti-B10 which is negative on H-2^d and reacts with H-2^b and Ia^b of the C57BL/6 mice. Serum 3 is a $(B10.A(4R) \times A.BY)F_1$ anti-B10.A(2R) which is negative on H-2^b and reacts with Ia^d of BALB/c. NT, Not tested.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two or three experiments.

cells were IF positive with a polyvalent anti-Ia antiserum (A.TH anti-A.TL) detecting Ia antigens of H-2^a origin. These findings suggest that the Langerhans cells of the transplanted skin were derived from a mobile pool of recipient cells.

Our second set of experiments was designed to assess more directly the origin of the Langerhans cells. Chimaeric animals were prepared by irradiating mice with 750–900 R and reconstituting with $2.5–3 \times 10^7$ allogeneic or semi-allogeneic bone marrow cells. All mice used in our studies were shown to be 'totally' chimaeric by the demonstration of H-2 and Ia antigens of the donor strain on spleen and peripheral blood cells. Table 2 demonstrates that after various periods of time following chimaerisation (BALB/c into C57BL/6) 20–45% of the Fc-IgG-bearing cells were of donor origin whereas the remainder of the Fc-IgG-bearing cells were of recipient origin. These studies also show that the bulk of the epidermal cells (keratinocytes) come from the recipient animal, as most of the epidermal cells were IF positive with the anti H-2^b (B10.D2 anti-B10) antiserum. In another strain combination $(B10 \times B10.A)F_1$ into B10.A a much larger percentage of Fc-IgG-bearing cells were found to be of donor origin 80–85 d after chimaerisation (Table 3). Here we determined that 68% [using a $(B10.A \times A)F_1$ anti-B10.D2 antiserum] to 80% [using a $(B10.A \times A)F_1$ anti-B10 antiserum] of the Fc-IgG-rosetted cells were of donor bone marrow origin. Electron microscopic studies of the Fc-IgG-bearing rosetted cells demonstrated that most of the cells had all the characteristics of Langerhans cells, including the unique cytoplasmic organelles (Fig. 1).

Taken together, these studies indicate that epidermal Langerhans cells are derived from and are continually repopulated by a mobile pool of precursor cells which for the most part originate in the bone marrow. The most likely precursor cell is the monocyte, as Langerhans cells exhibit similar, if not identical, surface marker characteristics to cells of the monocyte–macrophage series. None of our chimaera studies produced total replacement of epidermal LC by donor precursor cells. This may be due to the fact that residual host precursor monocytes remain in the bone marrow at the dose of irradiation used, even though the peripheral lymphoid cells are completely replaced. This possibility is under study. The demonstration of the non-total replacement of the monocyte pool may be important for the interpretation of mechanistic studies using allogeneic chimaeras.

Langerhans cells probably subserve functions of the Ia-rich relatively non-phagocytic macrophages and may not only function in an antigen-presenting capacity for exogenous allergens but also represent the 'passenger leukocyte' which is thought to induce graft rejection. In this regard, Steinmuller has reported sensitisation of animals by transplantation of skin from chimaeras[10]. In these experiments, skin was transplanted from chimaeras (A recipient, $(A \times B)F_1$ donor) on to strain A mice. The grafts were accepted, but when skin was then transplanted from B animals on to the same A mice, the second grafts were rejected in an accelerated fashion. The most likely source of the B antigens in the chimaera skin was the haematopoietically derived cells from the donor. Our data indicate that the chimaeric skin becomes rich in donor Ia because of the appearance of donor Langerhans cells in the epidermis. It is likely that these cells have an important role in allogeneic graft rejection. Such conclusions are consistent with our previous studies

Table 3 Detection of major histocompatibility alloantigens on epidermal cells of chimaeric mice

Donor	Recipient	Antisera: Serum 1 (anti-H-2^b)		Serum 2 (anti-H-2^a)		Serum 3 (anti-Ia^b)		Serum 4 (anti-Ia^a)	
		%IF +ve*	%Rosette +ve†	%IF +ve	%Rosette +ve	%IF +ve	%Rosette +ve	%IF +ve	%Rosette +ve
$(B10 \times B10.A)F_1$ (H-$2^{a/b}$, $I^{a/b}$)	B10.A(H-2^a, Ia^a)	6.3‡	80	91	81	4.5	68	5.1	78
Controls									
A/J(H-2^a, Ia.7)		0	0	95	100	0	0	3.8	100
C57BL/6(H-2^b, Ia.8)		75	96	0	0	3.6	75	0	0

The animals were tested 80–85 d after chimaerisation. The antisera used have been extensively characterised by cytotoxicity and absorption analysis on spleen and lymph node cells. Serum 1 is a $(B10.A \times A)F_1$ anti-B10 which is negative on H-2^a and reacts with H-2^b and Ia^b. Serum 2 is an A.BY anti-A/J which is negative on H-2^b and reacts with H-2^a. Serum 3 is a $(B10.A \times A)F_1$ anti-B10.D2 which is negative on H-2^a and reacts with the Ia^b specificity Ia.8. Serum 4 is an $(A.TH \times B10.S)F_1$ anti-B10.HTT and is negative on H-2^b and reacts with the Ia^a specificity Ia.7.

* Per cent of viable cells which fluoresce.

† Per cent of Fc-IgG-receptor-positive cells (Langerhans cells) which fluoresce.

‡ Each result represents the mean of two experiments.

demonstrating the antigen-presenting and allogeneic-stimulatory capacities of Langerhans cells[5].

The chimaeric animals were provided by Drs R. Almarez and A. Singer.

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Corneal allografts fail to express Ia antigens

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Although the surface determinants encoded by class I genes of the major histocompatibility complex (murine K/D; human HLA-A/B/C) are expressed on virtually all nucleated cells, the molecular products of class II genes (murine I, human HLA-D/DR) are expressed only on B lymphocytes, macrophages, sperm and certain subsets of T lymphocytes, especially suppressor cells[1,2]. Controversy exists concerning the expression of class II antigens on cells of the epidermis. On the one hand, Hammerling *et al.*[3] and Frelinger *et al.*[4] have used serological methods to detect Ia antigens on epidermal cells. On the other hand, fluorescein isothiocyanate-conjugated antisera have been used to show that human and guinea pig class II alloantigens are only expressed on Langerhans cells in the epidermis[5–7]. Recently, Rowden *et al.*[8] have convincingly demonstrated that Ia antigens are expressed exclusively on Langerhans cells within murine epidermis. We have now shown that cornea, which is an epidermal tissue devoid of Langerhans cells, does not express Ia antigens.

Langerhans cells have been found to be closely associated with the induction and expression of delayed cutaneous hypersensitivity reactions elicited by chemical contactants[9,10], but not all cutaneous sites are equally endowed with these cells, the density of which varies considerably in skin from mouse ears, foot pad and body wall[11]. Similarly, we have recently demonstrated marked variation in Langerhans cell-surface density using an ATPase staining method in mice[12]. The most dramatic difference from body wall skin, which contains approximately 800 ± 200 Langerhans cells per mm^2, was found in the cornea which is completely free of ATPase-positive, dendritic cells, that is, Langerhans cells. This observation is consistent with a previous series of studies[12–15]. Recently, it has been claimed that Langerhans cells, as defined by surface Ia antigens, are present at the periphery of the cornea and the limbus[16]. We also find ATPase-positive cells at the corneal limbus. In the grafting experiments described below, we took special care to prepare corneal grafts by severely cutting away peripheral tissues, including the limbus.

To determine whether alloantigens encoded by the I region of the murine H-2 complex can be expressed functionally in epidermal tissue if Langerhans cells are not present we placed corneal grafts on prepared beds on thoracic walls of recipient mice that differed from their corneal graft donors at the K region alone, at combined K and I regions, and at the I region alone. These corneal allografts were observed for evidence of rejection over a 45-d interval. Recipients were then rechallenged with conventional body wall skin grafts syngeneic to the original corneal allografts. Median survival times (MSTs) were calculated and compared with appropriate controls to determine whether first- or second-set patterns of rejection had taken place.

The fate of syngeneic corneal grafts must first be described. All grafts were carefully trimmed of scleral and limbic tissues and placed, endothelial-side down, on the raw sub-dermal surface. Each graft bed received three corneal grafts. The site was covered with Vaseline-impregnated gauze and wrapped with plaster of Paris bandage. Plaster casts were first removed for graft inspection 8 d later. At that time, three circular, transparent corneal grafts were usually found. When one of the grafts had been placed strategically over a subcutaneous blood vessel of significant size, blood could be seen coursing through the vessel directly beneath the transparent graft. Corneal grafts retained their transparency so long as they were covered and dressed with the Vaseline gauze. After exposure to room air, corneal transparency gradually gave way over several days to translucency which then persisted indefinitely. Thereafter, corneal grafts were identifiable by their smooth surface, and by the slightly dome-shaped contour due to the inherent structure of the corneal stroma. Healthy autografts of this type have been observed beyond 60 d. There was very little wound contracture at these graft sites during the observation period.

The results of the allografting experiments described in outline above are presented in Table 1. H-2-congeneic strains B10.A and B10.AQR differ at the K region: B10.A possesses the K^k allele, whereas B10.AQR possesses the K^q allele. The MST of first-set B10.A body wall skin grafts placed in B10.AQR recipients was 16 ± 1.1 d. The MST of B10.A corneal allografts on B10.AQR recipients was similar, 13 ± 1.1 d. During their early course the gross appearance of these corneal allografts was quite similar to that of syngeneic corneal grafts. However, 1–2 d before rejection, the graft beds became acutely inflamed; the surface epidermis of the grafts developed hyperkeratotic changes and then quickly sloughed, to be replaced by a dried eschar. Within the next 24–48 h, wound contracture began and by 5 d, only a small, crusted wound, a few millimetres in diameter, remained.

Table 1 Comparative sensitising capacities of allografts prepared from cornea and body wall skin

	First-set graft					Second-set graft			
Recipient	Donor	H-2 Disp.	Graft source	*n*	MST	Donor	H-2 Disp.	*n*	MST
1. B10.AQR	B10.A	K^k	Body wall	13	16.0 ± 1.1	B10.A	K^k	13	9.0 ± 1.1
2. B10.AQR	B10.A	K^k	Cornea	11	13 ± 1.1	B10.A	K^k	11	9.0 ± 1.2
3. A.TH	A.TL	I^k	Body wall	10	16 ± 1.3	A.AL	K^kI^k	10	9.5 ± 1.3
4. A.TH	A.AL	K^kI^k	Body wall	10	14.8 ± 1.1	A.AL	K^kI^k	10	9.0 ± 1.0
5. A.TH	A.TL	I^k	Cornea	13	>45	A.AL	K^kI^k	13	14.8 ± 1.1
6. A.TH	None					A.AL	K^kI^k	8	15.0 ± 1.1

All second-set grafts were prepared from body wall skin. H-2 disp. refers to H-2 differences between donor and recipient. I = ABJEC subregions plus the S and G regions of H-2. MST = Median survival time in days, ±1 s.d.

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H-2-congeneic strains A.TL and A.TH differ from each other across the entire I region of H-2, plus the S and G regions: A.TL expresses the I-ABJECSG alleles of k, whereas A.TH expresses comparable alleles of the s halotype. First-set body wall skin grafts from A.TL on A.TH recipients were rejected acutely, MST 16 ± 1.3 d (Table 1, line 3). In contrast, A.TL corneal allografts survived on A.TH recipients beyond 45 d (line 5). No grafts were rejected. In fact, the appearance of these grafts throughout the observation interval was virtually identical to that of syngeneic corneal grafts. These data indicate that epidermis deficient in Langerhans cells is similarly deficient in expression of alloantigenic products of I-region genes and support the claim that only Langerhans cells within the epidermis express I-region determinants in an immunogenically important way.

This interpretation was strengthened by the findings of the next experiments. Recipients of body wall and corneal first-set allografts were regrafted with body wall skin prepared from donors expressing the alloantigens putatively present on the first-set grafts. Thus, when B10.AQR animals were rechallenged with B10.A body wall skin grafts, they rejected these grafts in an accelerated, second-set manner, indicating that body wall skin and cornea are equally able to sensitise to the alloantigens of the K region (Table 1, lines 1, 2). Similarly, A.TH animals grafted first with A.TL body wall skin rejected test grafts of A.AL skin bearing identical I^k determinants in a second-set fashion (MST of 9.5 compared with 16 d, Table 1, line 3). However, when A.TH recipients of I-region-disparate A.TL corneal grafts were challenged with body wall skin from A.AL donors (expressing the same I region disparity), the test grafts were rejected in first-set fashion, indicating that these animals had not been sensitised by their prior experience with the corneal graft (MST of 14.8 and 15 compared with 9.0 d, Table 1, lines 5–6). We conclude that corneas in which the epithelium is essentially devoid of a resident Langerhans cell population are unable to display class II alloantigens in a manner capable of inciting transplantation immunity. We recognise that, as the corneal allografts failed to sensitise to Ia alloantigens, the stromal elements and endothelium of these grafts must also be unable to express these antigens effectively.

Our findings have immediate relevance to clinical corneal transplantation. Even without tissue typing to assist donor selection, corneal allografts have been surprisingly, although not regularly, successful in man[17]. Our results suggest that deficiency of Langerhans cells in the cornea may be important in this. HLA-D/DR alloantigens are potent transplantation immunogens in man. If, by extrapolation from the mouse data, human corneas fail to express D/DR determinants, then the alloantigenic burden of cornea is significantly less. Perhaps for the success of corneal transplants in man, matching for HLA-A, B, C may be sufficient. A recent report indicates that donor–recipient matching for HLA-A, B, C corresponds to improved corneal allograft survival[18].

The absence of Langerhans cells from the cornea has other interesting implications. It has been demonstrated that, shortly after vaccinia virus vaccination in man, virtually all viral particles become associated with epidermal Langerhans cells[19]. These cells have been ascribed the general property of antigen processing; perhaps the processing and/or handling of cutaneous viral infections is also a function of the dendritic Langerhans cell. Indolent and persistent infections of corneal epithelium often caused by herpes simplex virus may be another expression of the virally related function of this interesting epidermal cell type. The absence of Langerhans cells from the cornea (assuming that they are important in defence against viral infection) would render this epidermal tissue especially vulnerable to persistent and chronic virus infection.

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A defect in the antigen-presenting function of macrophages from neonatal mice

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The newborn mammal has a limited ability to mount an immune response[1–5]. As the individual matures, the potential for immunological reactivity develops and expands. During the early period of relative immune incompetence, tolerance to some foreign antigens is easily induced[6,7]. It is thought that the individual may be acquiring tolerance to self antigens during this period, hence its importance for immunological homeostasis. The contribution of mononuclear phagocytes to the initiation of immune responses is now well established. These phagocytes are essential for the development of various lymphocyte functions, in particular, those of the T lymphocytes. Most T-lymphocyte activities require that the macrophage take up and 'present' the antigen in a process modulated by the I region of the major histocompatibility gene complex (for review see ref. 8). Although some earlier studies[5,9–13] have indicated deficient macrophage function in the neonate, precise definition of the putative deficiency is lacking. We show here that macrophages from neonatal mice present antigen poorly and that this can be correlated with the small number of macrophages that bear Ia antigens.

In our tissue culture assay system, T lymphocytes from mice immunised to *Listeria monocytogenes* were cultured with macrophages which had taken up heat-killed *Listeria* organisms. The macrophages were always obtained from non-immune mice. It had been previously established that effective interaction between T lymphocyte, macrophage and *Listeria* resulted in T-lymphocyte proliferation[14]. Proliferation of the *Listeria*-immune T lymphocytes was antigen specific and required uptake of *Listeria* by a subpopulation of macrophages bearing Ia antigen and homology between T lymphocyte and macrophage at the I-A region of the major histocompatibility gene complex. This assay system is similar to other systems[8,15] using soluble protein antigens and measures an antigen-presentation function of the macrophage regulated by the major histocompatibility gene complex.

The data in Table 1 demonstrate that culturing *Listeria*-immune T lymphocytes from adult (2–3-month-old) mice with *Listeria*-pulsed macrophages also from adults resulted in T-lymphocyte proliferation. A weak proliferative response was observed when the same number of macrophages from neonatal mice was substituted for the macrophages from adults. Direct microscopic counts established that equal numbers of adherent

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Table 1 Comparison of adult immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages from C57BL/6J mice of different ages

Listeria-immune T cells	Age of macrophage donor	Pulse of *Listeria*	c.p.m.
+	No macrophages	0	1,024 ± 210
+	8–12 weeks	0	577 ± 28
		+	43,367 ± 1,201
+	4 days	0	647 ± 123
		+	1,947 ± 170
+	10 days	0	492 ± 20
		+	3,699 ± 981
+	15 days	0	670 ± 109
		+	4,803 ± 1,451

Macrophages were obtained from the peptone-elicited peritoneal exudate of non-infected C57BL/6J mice of various ages. Sterile proteose peptone, 10% (75 μl per g body weight) was injected intraperitoneally (i.p.), and the resulting exudate collected 4 d later by lavage with Hank's balanced salt solution containing 2% fetal calf serum (FCS), 10 mM HEPES and 10 $U l^{-1}$ heparin. Peritoneal exudate cells (10^4) were incubated on flat-bottom 1.0 × 0.7-cm^2 Linbro wells for 1 h, and then nonadherent cells were removed by washing the wells three times. The adherent cells were pulsed with heat-killed *Listeria for* 1 h. Unbound *Listeria* was removed by washing the wells five times, after which 4×10^5 *Listeria*-immune T lymphocytes were added to each well in 200 μl of RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol. The immune non-adherent T lymphocytes were cultured with the *Listeria*-pulsed macrophages for 4 d at 37°C in humidified 5% CO_2. Each well then received 0.2 μCi ^{3}H-thymidine, and trichloroacetic acid-precipitable counts were measured 18 h later. Results indicate the mean of three cultures with the s.e.m. Further technical details are given in ref. 14. The *Listeria*-immune T lymphocytes were isolated from adult C57BL/6 mice injected i.p. with 10^4–10^5 live *Listeria*. Seven days after infection, the mice received 1.5 ml of sterile 10% proteose peptone i.p., and the resulting exudate was collected 3 d later. The T lymphocytes were isolated by removing macrophages through adherence on plastic dishes and nylon-wool filtration. Other experiments from this laboratory have firmly established that T lymphocytes cultured without macrophages but with *Listeria* continuously show no proliferation or a modest proliferative response never exceeding about one-quarter of that shown with macrophage-bound *Listeria*[14]. By doing the experiment, as above, pulsing the macrophages with *Listeria* before addition of T cells, we minimise any possible effect of residual macrophages in the T-cell population.

cells from neonatal and adult mice were being compared. The adherent cell population from neonates and adults had the same composition—greater than 99% macrophages as determined by morphology and positive, nonspecific esterase staining. Furthermore, the macrophages from adult and neonatal mice had exactly the same ability to take up *Listeria* and similar distributions of Fc and C3 receptors.

Figure 1 shows that only optimal numbers of macrophages from adults, 10^4–3×10^4 per microwell, stimulated immune T-cell proliferation. Macrophages from neonatal mice weakly stimulated T-lymphocyte proliferation regardless of the number of macrophages in the microwells. Furthermore, increasing the amount of *Listeria* given to the macrophages from neonatal

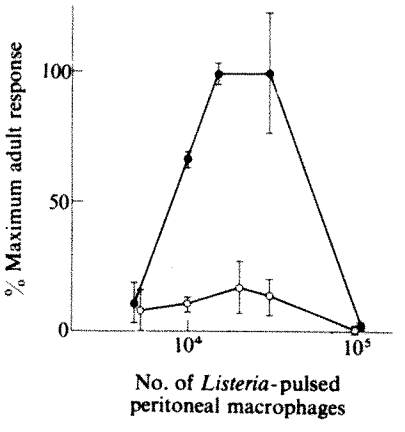


Fig. 1 Immune T-lymphocyte stimulation by antigen-pulsed macrophages as a function of the number of macrophages. The assay of *Listeria*-immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages is described in Table 1 legend. Macrophages from neonatal (○) or adult (●) mice were pulsed with 10^7 *Listeria* for 1 h. The figure summarises three experiments.

animals did not overcome their deficient ability to stimulate immune T-lymphocyte proliferation (data not shown).

Figure 2 shows that the ability of macrophages to interact effectively with T lymphocytes did not occur until 3–4 weeks after birth, approximately the time of weaning. In each of nine experiments, peritoneal macrophages were taken from C57BL/6J mice of various ages, given *Listeria*, and used to stimulate immune syngeneic T-lymphocyte proliferation. The deficiency detailed above also applied to neonatal splenic adherent cells (not shown). The deficiency was also found in macrophages taken from neonatal mice of a different strain, the A/St strain ($H\text{-}2^a$ haplotype).

We have also investigated effects of T-lymphocyte–macrophage interaction other than proliferation. Macrophages secrete a 15,000-molecular weight protein mitogenic for thymocytes[14,16]. This secretion is optimal when the macrophage, antigen and T lymphocyte interaction occurs with I–A homology between the two cells. Although macrophages from adults secreted the mitogen when mixed with *Listeria*-immune T lymphocytes and *Listeria*, this was not the case with macrophages from neonatal animals (not shown).

There are several possible explanations for the deficient ability of macrophages from neonatal mice to stimulate T-lymphocyte proliferation. First, a possible contamination of the macrophage population with suppressor T cells seems unlikely because of the purity of the macrophage populations. Furthermore, treatment of macrophages with anti-Thy-1.2 and complement did not change the results. Second, a possible nonspecific suppressive influence of macrophages from neonatal mice on lymphocyte proliferation[17] was investigated by mixing macrophages from neonatal animals with syngeneic splenic T lymphocytes stimulated with concanavalin A. Table 2 shows that the macrophages from the neonatal mouse did not suppress the concanavalin A response. In several experiments, we have found that macrophages from adults can stimulate *Listeria*-immune T-lymphocyte proliferation in cultures containing equal numbers of macrophages from neonatal animals. In one example, the response of immune T lymphocytes to 10^4 macrophages from adults, 10^4 from neonatal animals and a mixture of 10^4 cells of both was 19,218, 3,227 and 16,950 c.p.m., respectively.

The inability of macrophages from neonatal mice to stimulate T-lymphocyte proliferation effectively may be due to the small number of Ia-bearing macrophages in the neonatal animals (Table 3). The macrophage population fron neonatal mice was deficient in a subpopulation carrying surface Ia antigen. This observation was made with various antisera–target cell combinations. The appearance of Ia-positive macrophages in ontogeny varied from experiment to experiment, perhaps reflecting uncontrollable environmental conditions. It has been

Table 2 Macrophages from neonatal and adult mice do not inhibit concanavalin A-stimulated splenocyte proliferation

Nylon-wool-filtered splenocytes	Age of macrophage donor	Concanavalin A	c.p.m. Expt 1	c.p.m. Expt 2
+	—	0	2,762 ± 87	590 ± 27
+	—	+	53,469 ± 4,736	25,584 ± 996
0	9–11 d	+	348 ± 49	—
+	9–11 d	0	4,540 ± 591	—
+	9–11 d	+	61,732 ± 1,922	30,923 ± 1,821
0	10–12 weeks	+	272 ± 35	—
+	10–12 weeks	0	7,000 ± 860	—
+	10–12 weeks	+	68,270 ± 2,367	41,682 ± 1,469

Macrophages (10^4 per well) were collected from neonatal or adult mice and pulsed with 10^7 heat-killed *Listeria* as described in Table 1 legend. Splenocytes were collected from 2–3-month-old C57BL/6J mice and enriched for T lymphocytes by passage over nylon-wool columns. Nylon-wool-filtered cells (4×10^5) were placed in 1.0 × 0.7 cm^2 Linbro wells containing the *Listeria*-pulsed macrophages. Concanavalin A (4 μg ml^{-1}) was added to the wells. After 4 d of culture, each well received 0.2 μCi ^{3}H-thymidine, and trichloroacetic acid-precipitable counts were measured 18 h later. Similar results were obtained when 5×10^4 instead of 10^4 macrophages per microwell were used.

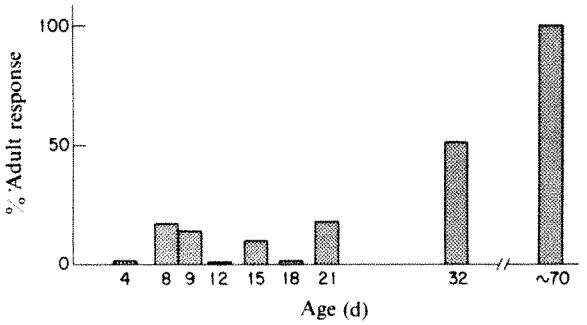


Fig. 2 Ontogeny of immune T-cell stimulation by *Listeria*-pulsed macrophages. Table 1 legend described the assay system for measuring immune T-lymphocyte stimulation by *Listeria*-pulsed macrophages taken from C57BL/6J mice of various ages. The immune T-cell stimulation by macrophages from mice of various ages is expressed as a percentage of the immune T-cells stimulation by macrophages from adult mice. The results were obtained from nine separate experiments.

established in the adult that only Ia-bearing macrophages present *Listeria* and that the cooperation between macrophage and T lymphocyte which allows antigen-driven T-lymphocyte proliferation requires homology between macrophage and T lymphocyte at the I-A region of the major histocompatibility gene complex[14,16]. Extrapolating from these data, one would predict that a macrophage population with a few Ia-positive cells would present antigen poorly, and this may be the case for the neonatal animal. Whether other factors in the neonatal macrophage population are involved is not known.

Table 3 Detection of Ia antigens on peptone-elicited macrophages

Expt	Strain of macrophage	Age of macrophage donor	Cytotoxic index (% dead)
1	C57BL/6J	8–12 weeks	29
		7 d	9
		10 d	3
2	C57BL/6J	8–12 weeks	25
		8 d	2
		25 d	2
3	C57BL/6J	8–12 weeks	40
		8 d	2
		23 d	29
		38 d	16
4	A.TL	8–12 weeks	29
		10 d	8
		23 d	9
5	C57BL/6	8–12 weeks	28
		12 d	0

Macrophages were isolated from peptone peritoneal exudates by adherence and then incubated for 1 h in antisera at 0 °C in flat-bottom 10-mm culture plates. After washing the macrophages at 0 °C, these cells were incubated in a mixture of 6% rabbit and 4% guinea pig complement for 1 h at 37°C. The sera had been absorbed extensively with mouse spleen cells to remove natural anti-membrane antibodies. The complement was washed away, and the macrophages were incubated in 0.4% trypan blue in normal saline at room temperature for 15 min. Dead and living cells were scored under a Nikon inverted microscope. Neither dead (trypan-blue-positive) nor living (trypan-blue-negative) macrophages were lost from the microwells during the antiserum-complement treatment. The number of dead macrophages following treatment with normal mouse serum (NMS) plus complement was never greater than 5%. The results are expressed as the cytotoxic index: ([% Ia positive − % NMS positive] × 100)/(100 − % NMS positive). The A.TH-anti-A.TL antiserum used in expts 1 and 2 has been previously used[14] and shown to be Ia specific by appropriate absorptions. Used against C57BL/6J macrophages, this antiserum will detect Ia specificity 3 (ref. 25). The A.TH-anti-A.TL antiserum used in expts 3 and 5 (Cedarlane Laboratories) had also been previously standardised. The antiserum used in expt 4 was raised by injecting (LP × A.TH)F_1 mice with (LP × A.TL)F_1 and C157 splenocytes and was absorbed with B10.7R spleen cells. The antiserum should detect Ia specificities 1, 2 and 7 (refs 25, 26). This antiserum did not kill control C57BL/6J spleen cells, which do not have Ia specificities 1, 2 or 7. None of these antisera killed control nylon-wool-purified A/St or C57BL/6J splenic T lymphocytes which lack significant amounts of Ia. All antisera were used at an optimal dilution. The cytotoxic index of macrophages taken from mice of similar ages varies from experiment to experiment. This may be attributed to variation in the rate of development of individual litters.

Our study demonstrates that macrophages from neonatal mice do not present antigen efficiently and that this can be correlated with the small number of macrophages bearing Ia antigens. These results allow us to speculate about the two major characteristics of the neonatal immune response. First, with few antigen-presenting macrophages, the T-lymphocyte responses regulated by the major histocompatibility complex (helper, cellular immunity) would not be induced. This would contribute to the limited immune response capacity of the neonatal mouse. Second, interaction of antigen directly with T lymphocytes in the absence of Ia-positive macrophages may induce antigen-specific suppressor T lymphocytes[18,19]. This would suggest another mechanism for control of self-recognition and the ease of tolerance induction seen in the newborn. Of course, the macrophage is not the only immature cell in the neonatal animal. B lymphocytes[20–23] and T lymphocytes[5,24], each with unique properties during early life, have been described and also contribute to the special characteristics of the neonatal immune response.

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Acetylcholine receptor-controlled ion fluxes in membrane vesicles investigated by fast reaction techniques

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The ability of a nerve cell to integrate its response to chemical signals (neurotransmitters) arriving from many other cells is central to the function of the nervous system. This integration process depends on the ability of the neurotransmitters to bind to specific receptors which modulate the flow of specific inorganic ions through the cell membrane, and on the ability of the cell to transmit a (chemical) signal to an adjacent cell[1–3] only if the resulting change in transmembrane potential is of appropriate sign and magnitude. The controlling sign and magnitude of the cell transmembrane potential can be predicted from measurement of the rates of movement of specific inorganic ions across the membrane[4–6] and their dependence on neurotransmitter concentration. However, there have been two main

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obstacles to the development of a suitable preparation for such measurements. First, Kasai and Changeux[7] obtained a membrane vesicle preparation from the electric organ of *Electrophorus electricus* (electroplax vesicles) in which acetylcholine receptor-controlled fluxes of inorganic ions could be observed, but the flux rates seemed too slow to be of physiological significance[8]. We have shown that these slow flux rates were associated with a large fraction of the vesicle preparation which did not respond to acetylcholine and yet dominated the measurements[9]. We then accomplished the separation of the receptor-controlled fluxes from other processes[9,10]. Second, the receptor-controlled flux was found to be biphasic, with an initial phase too fast to be measured by available techniques[11]. We have now succeeded in applying a quench flow technique[12] to the determination of the flux rate of specific inorganic ions across vesicle membranes in the millisecond time region, a time resolution sufficient for measuring the initial phase of receptor-controlled fluxes with these vesicles.

The receptor-controlled fluxes were characterised by obtaining three different kinetic measurements using the quench flow technique. Figure 1*a* shows rapid receptor-mediated flux of $^{86}Rb^+$ into the vesicles in the presence of 1 mM carbamylcholine, a stable analogue of acetylcholine. The influx was stopped after various times by mixing the reaction solution with (+)tubocurarine solution to give a final concentration of 10 mM. The $^{86}Rb^+$ content of the vesicles was determined by a Millipore filter assay as previously described[7]. Half of the $^{86}Rb^+$ influx occurs in 160 ms and the process is complete within 1 s, no further influx being observed at least within 30 s. The data in Fig. 1*b* demonstrate that receptor-controlled flux is affected by preincubation of the vesicles with 1 mM carbamylcholine. After the various preincubation times indicated, the vesicles were mixed with $^{86}Rb^+$ and influx was allowed to proceed for 6 s in the presence of 1 mM carbamylcholine. In vesicles which had not been preincubated with 1 mM carbamylcholine, influx was complete within this assay period. With vesicles which had first been preincubated with 1 mM carbamylcholine for 450 ms, influx was only 50% complete within the assay period. As Fig. 1*c* shows, a relatively slow rate of flux is observed after preincubation of the vesicles with 1 mM carbamylcholine for 10 s. After this preincubation, influx of $^{86}Rb^+$ in the presence of 1 mM carbamylcholine was measured at the times indicated. The final concentration of $^{86}Rb^+$ in the vesicles was the same as in the experiment shown in Fig. 1*a*, but ~25 s were required for half the $^{86}Rb^+$ influx to occur, that is, 150 times longer than when the vesicles had not been preincubated with carbamylcholine.

The model in Fig. 2 was used to evaluate the data shown in Fig. 1. It is based on the observation that acetylcholine receptor-mediated changes in the transmembrane potential of nerve and muscle cells often last less than 1 s, even though acetylcholine or carbamylcholine remains. This decrease in response to neurotransmitter is called desensitisation. To explain the phenomenon, Katz and Thesleff[13] suggested that the receptor exists predominantly in an active conformation and that this conformation is converted to an inactive form by these compounds. On the basis of the observation that receptor-controlled flux consists of an initial fast phase which is followed by a slow phase[11], we suggested that the active conformation is associated with a large flux rate constant (J_1, Fig. 2), and the inactive conformation with a much smaller one (J_2, Fig. 2).

The terms of the integrated rate equation pertaining to the model (Fig. 2) are readily evaluated from kinetic measurements because the two phases of the flux occur in separate time regions (Fig. 1). (1) At high concentrations of carbamylcholine the flux associated with the active form of the receptor, indicated by △ in Fig. 2, dominates the kinetics. The solid line in Fig. 1*a* was computed using equation (1) (Fig. 2), a β value of 1.5 s^{-1} obtained from the data in Fig. 1*b*, and a value for $J_1(L_0/L_0+K_1)$ of 4.8 s^{-1}, which was obtained by a curve-fitting procedure. (2) The disappearance of the fast flux observed in Fig. 1*b* is a measure of the disappearance of the active form of the receptor. β contains the rate constants, k_{12}, k_{23}, k_{34} and k_{43}, for the

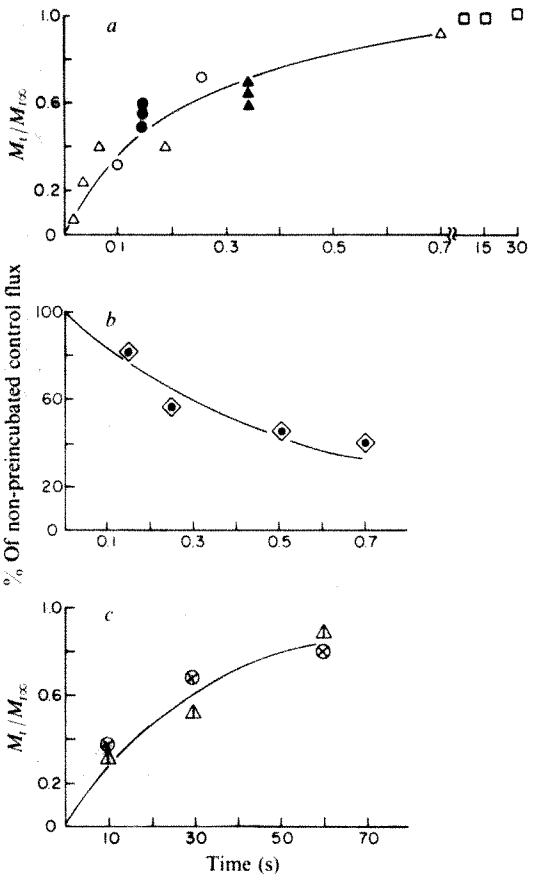


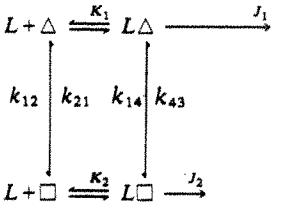
Fig. 1 Acetylcholine receptor-controlled $^{86}Rb^+$ flux in electroplax membrane vesicles, *p*H 7.0, 1 °C. *a*, Flux of $^{86}Rb^+$ into vesicles in the presence of 1 mM carbamylcholine. $[M^+]$ is proportional to the concentration of $^{86}Rb^+$ in the vesicles. The subscript t indicates the time of measurement. *b*, Inactivation of the acetylcholine receptor-controlled $^{86}Rb^+$ influx by preincubation of the vesicles with 1 mM carbamylcholine for various periods of time, after which influx was allowed to proceed for 6 s in the presence of $^{86}Rb^+$ and 1 mM carbamylcholine. The ordinate gives the results as a percentage of the flux measured when carbamylcholine was absent during preincubation. *c*, Flux of $^{86}Rb^+$ into vesicles in the presence of 1 mM carbamylcholine, after preincubation of the vesicles for 10 s with 1 mM carbamylcholine. The different symbols refer to different eel preparations: solid symbols represent single measurements; the symbols in *b* represent the average values obtained in four different experiments using membrane preparations from four different eels. The quench flow apparatus has been described by Fersht and Jakes[12]. Vesicles (800 μg membrane protein per ml) were equilibrated for at least 12 h with *E. electricus* Ringer solution[18] (169 mM NaCl, 5 mM KCl, 3 mM $CaCl_2$, 1.5 mM $MgCl_2$, 1.5 mM phosphate buffer, *p*H 7.0). In the influx experiments the vesicles were mixed with an equal volume of eel Ringer solution containing either $^{86}Rb^+$ (100 μCi ml^{-1}) or $^{86}Rb^+$ and 2 mM carbamylcholine. After the predetermined time 2 volumes of the above solution were mixed with 1 volume of 30 mM (+)tubocurarine chloride which quenched the reaction. The $^{86}Rb^+$ content of the vesicles was measured using a Millipore filter assay[7]. The effectiveness of quenching was demonstrated by mixing a solution containing vesicles, carbamylcholine and (+)tubocurarine with a solution containing $^{86}Rb^+$ and observing no influx in 5 s, and also by sequentially mixing the vesicles with carbamylcholine and $^{86}Rb^+$ and then with (+)tubocurarine and observing no influx. In the desensitisation experiments the vesicles were mixed with an equal volume of 2 mM carbamylcholine or eel Ringer solution. After the predetermined preincubation period 2 volumes of the above solution were mixed with 1 volume of $^{86}Rb^+$ and 1 mM or 3 mM carbamylcholine (the final concentration always being 1 mM), and the mixtures incubated for 5 s before Millipore assay. To ensure that vesicle breakage was not significant, the total $^{86}Rb^+$ influx measured in the quench flow apparatus was compared with the total influx measured outside the apparatus. The method for isolating the vesicles, the kinetic techniques which allow measurement of the carbamylcholine-induced fluxes of inorganic ions without interference from other fluxes, and the sources of chemicals used, have been described elsewhere[9–11].

isomerisation of receptor conformations[14], and was evaluated from a linear plot of the data according to equation (2) with the concentration of the active form of the receptor at zero time, $[L\Delta]_{t=0}$, set equal to 100% (Fig. 1*b*). $[L\Delta]_t$ represents the concentration of the active receptor conformation at time t, and is obtained from the ordinate of the graph in Fig. 1*b*. The solid

line was computed using a value for β of 1.5 s^{-1}. (3) Preincubation of the vesicles with carbamylcholine for 10 s leads to a relatively slow $^{86}Rb^+$ influx which follows a first-order rate law. The solid line in Fig. 1*c* was computed using a value for $J_2(L_0/L_0+K_2)$ of 0.03 s^{-1}. We cannot determine *a priori* which receptor conformation gives rise to this slow flux. If we assume that it arises only from a small concentration of active receptor in an equilibrium mixture of active and inactive forms, $J_2 = K_{c2}J_1$.

Desensitisation of the acetylcholine receptor has previously been observed in flux experiments with electroplax membrane vesicles[14], and in electrophysiological measurements with intact electroplax cells[15,16]. The suggestion that these observations are due to the isomerisation of receptor conformations, each associated with very different efficiencies in mediating the equilibration of inorganic ions across the cell membrane, is supported by the data in Fig. 1. Furthermore, the overall rate constant for this process, β, measured in flux experiments with electroplax vesicles, is kinetically competent to account for the time course of the electrophysiologically observed desensitisation process in intact, single electroplax cells. The observation that the desensitised form of the receptor also allows inorganic ions to cross the membrane may account for several electrophysiological observations. The extent to which flux rates controlled by particular receptors perturb the transmembrane voltage depends on the extent to which they perturb the relative rates with which inorganic ions move through the cell membrane in the absence of the particular receptors[4-6,17]. The change in transmembrane potential due to the relatively slow rates associated with the desensitised form of the receptor, is expected to be particularly sensitive to these other fluxes of inorganic ions. Differences in the desensitisation behaviour of different cells, observed in electrophysiological experiments, may therefore reflect characteristics of the cell unrelated to the receptor.

The prediction of the sign and magnitude of the transmembrane potential of a cell, and therefore of the propagation of a signal in a variety of conditions, requires additional measurements. J_1 and J_2 can be evaluated from measurements of the effect of ligand concentration on the flux kinetics. Intrinsic flux rate constants can be determined from a knowledge of the effect of inorganic ion concentrations on J_1 and J_2, and of the number of receptor sites per vesicle and the internal volume of the vesicles. The results presented in Fig. 1 show that the technique which we have developed enable the required kinetic measurements to be made.



$$\ln\frac{[M^+]_t}{[M^+]_{t=0}} = -\frac{J_1L_0}{L_0+K_1}\left(\frac{1-e^{-\beta t}}{\beta}\right) - \frac{J_2L_0}{L_0+K_2}t \quad (1)$$

$$\ln\frac{[L\triangle]_{t=0}-[L\triangle]_t}{[L\triangle]_{t=0}} = -\beta t \quad (2)$$

$$\beta = \frac{k_{12}K_1}{L_0+K_1} + \frac{k_{34}L_0}{L_0+K_1} + \frac{k_{21}K_2}{L_0+K_2} + \frac{k_{43}L_0}{L_0+K_2} \quad (3)$$

$$K_{c2} = \frac{k_{43}}{k_{34}}, \qquad K_{c1} = \frac{k_{21}}{k_{12}} \quad (4)$$

Fig. 2 Minimum mechanism for acetylcholine receptor-controlled ion flux. The conformations referred to as active and inactive by Katz and Thesleff[13] on the basis of electrophysiological experiments are represented by △ and □, respectively. J_1 and J_2 are the rate coefficients for ion fluxes associated with the different receptor conformations (△ and □). Omitted from the scheme for simplicity are the equilibria between the open and closed channel forms of the receptor: ligand complexes, differences in which may be responsible for the differences between J_1 and J_2. K_1 and K_2 are the ligand dissociation constants pertaining to the two conformations. $[L\Delta]$ in equation (2) represents the concentration of the receptor: ligand complex and the subscript t refers to the time of measurement. β reflects the rate constants for the isomerisation of receptor conformations[11]. The integrated rate equation published previously[11] has been simplified, recognising that the two phases of the reaction occur in widely different time regions.

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A dominant role of thromboxane formation in secondary aggregation of platelets

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Aggregation of human platelets *in vitro* can occur in two phases—primary and secondary aggregation[1]. Primary aggregation is due to the direct interaction of the aggregating agent with its receptor[2,3], whereas secondary aggregation is associated with both the secretion of dense granule constituents, including the aggregating agents, serotonin and ADP (ref. 4) and the activation of a pathway for the conversion of arachidonic acid to prostaglandins (PGs)[5]. Both secondary aggregation and the associated secretion of dense granule constituents are contingent on the formation of PGG_2 and PGH_2 which can, in part, be transformed into thromboxane (TX) A_2 (refs 1, 3, 6, 7). TXA_2 is a potent aggregating agent and apparently the functionally active compound in the arachidonate pathway[8-10]. However, the precise role of TXA_2 in mediating secondary aggregation is a matter of controversy as it is not clear whether secondary aggregation can occur independently of secretion. We report here that platelets from cats with the Chediak–Higashi (CH) syndrome[11] aggregate with arachidonate in a cyclooxygenase-dependent manner and that biphasic aggregation produced by serotonin is accompanied by TXB_2 formation without detectable ADP secretion. These results demonstrate that secondary platelet aggregation can be mediated through thromboxane formation without participation of secreted ADP.

We have studied the aggregation and secretion of CH cat platelets and simultaneously measured TX formation. Most human patients and all animals, including the cats in this study,

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Table 1 Secretion of ADP and formation of TXB_2 during serotonin (5-HT)-induced aggregation of CH cat platelets

	Secreted ADP (μmol per 10^{11} platelets)*	TXB_2 Formed (nmol per 10^{11} platelets)†	
	12.5 μM 5-HT	12.5 μM 5-HT	50 μM 5-HT
CH Cat no. 1	<0.001	0.263	0.554
2	0.004	0.131	0.143
3	0.004	0.087	0.508
Normal	0.248	—	0.465‡

* Total adenine nucleotides (ATP and ADP) and ATP were determined in ethanol–EDTA extracts by a firefly luciferin/luciferase method[23].

† TXA_2 is unstable and rapidly decomposes to TXB_2 (ref. 7) which was measured by radioimmunoassay[24].

‡ TXB_2 formation was only assessed in platelets which exhibited serotonin-induced secondary aggregation.

with CH syndrome have an intrinsic platelet defect. In the one human patient studied[12] and all CH animals[13,14], there is a virtual absence of serotonin and stored adenine nucleotides. Therefore, aggregation of CH platelets should be independent of secreted ADP and serotonin.

Aggregation responses in platelet-rich-plasma (PRP) from CH cats were monitored with an aggregometer (Chrono-log Corporation). Figure 1 shows that arachidonate induced monophasic irreversible aggregation of CH cat platelets and that this aggregation was completely inhibited by the cyclooxygenase inhibitor indomethacin. Three minutes after the addition of arachidonate the extracellular ADP concentration of normal PRP was 2.14 μM whereas CH PRP contained less than 0.01 μM. These results indicate that metabolites of arachidonic acid can aggregate platelets in the absence of ADP or serotonin secretion and confirms the findings of other workers using rabbit

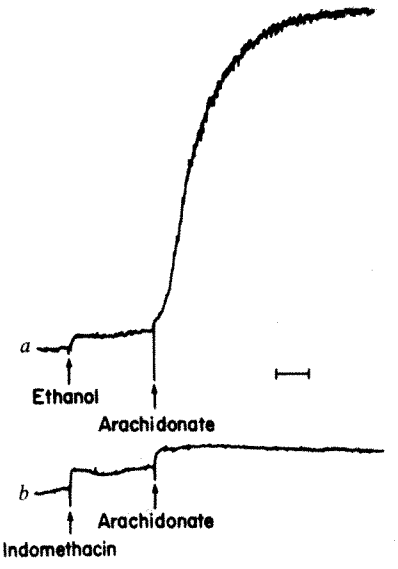


Fig. 1 Aggregation of platelets from CH cat no. 1 to 0.5 mM arachidonate following absolute ethanol treatment (20 μl ml^{-1}) (*a*) and its inhibition by 20 μM indomethacin in absolute ethanol (20 μl ml^{-1}) (*b*). Blood samples were withdrawn from the jugular vein from fasted cats anaesthetised with thiamylal sodium. Sodium citrate was used as the anticoagulant (1 part citrate: 9 parts blood). PRP was prepared by centrifugation at 160*g* at room temperature, and the platelet counts adjusted to 400,000 per μl using autologous platelet-poor plasma (PPP). Scale bar represents 20 s and the aggregation response of *a* represents 95% of change in light transmission between PRP and PPP. Arachidonate-induced aggregation of feline CH platelets was also inhibited by aspirin (20 μM) in sodium acetate, and platelets from normal cats gave monophasic aggregation with 0.5 mM arachidonate (data not shown).

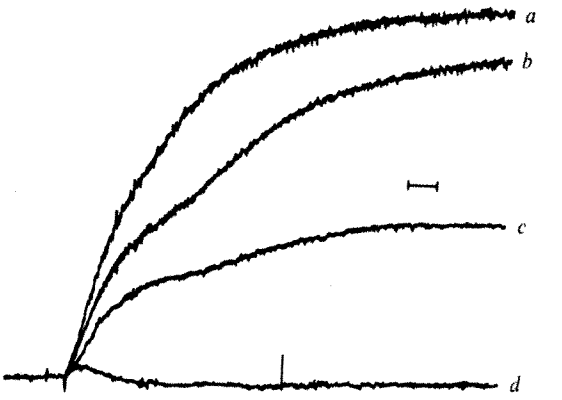


Fig. 2 Aggregation response of platelets from CH cat no. 1 to serotonin. *a*, 50; *b*, 12.5; *c*, 3; *d*, 1.5 μM. The scale bar represents 20 s and the aggregation response of *a* is 90% of the change in light transmission between PRP and PPP.

platelets depleted of the constituents of dense granules[15] and human platelets with nucleotide storage pool deficiency[16].

Serotonin is a potent inducer of the aggregation of normal cat platelets[17]. The aggregation is biphasic[17] and associated with nucleotide secretion (Table 1). Serotonin also induced biphasic aggregation of CH cat platelets (Fig. 2) which was not accompanied by ADP secretion (Table 1). With increasing concentrations, platelets exhibited first primary reversible aggregation, then biphasic aggregation and finally, monophasic irreversible aggregation. This finding clearly demonstrates that secondary aggregation may occur by processes independent of dense granule secretion.

The serotonin-induced aggregation of CH cat platelets was progressively inhibited by increasing concentrations of three serotonergic receptor antagonists, methysergide, SQ-10,631 and cyproheptadine (Fig. 3). Therefore, both the primary and secondary phases of aggregation seem to depend on the interaction between serotonin and its specific platelet receptor[2,3]. Although the impulse for the secondary wave of aggregation may have been initiated by the platelet–platelet contact which occurs during serotonin-induced primary aggregation, this seems unlikely as ADP induced primary, but not secondary, aggregation of CH cat platelets (data not shown).

The serotonin-induced secondary aggregation of CH cat platelets required arachidonic acid metabolism. When the formation of PGs was inhibited by treating CH cat platelets with either aspirin or indomethacin, secondary aggregation was abolished (Fig. 4). Furthermore, serotonin caused untreated

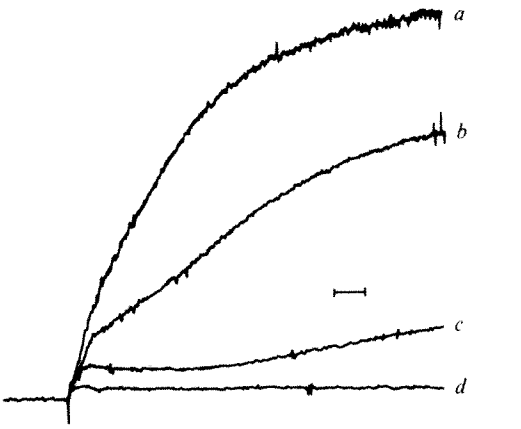


Fig. 3 Aggregation of platelets from CH cat no. 1 to 50 μM serotonin in the presence of increasing cyproheptadine concentrations: *a*, 0.31; *b*, 0.63; *c*, 1.25; *d*, 10 μM. Scale bar represents 20 s. Aggregation responses of *a*–*d* were, respectively, 88, 62, 16 and 1% of the change in light transmission between PRP and PPP. Similar aggregation curves were obtained with both methysergide and SQ-10,631 (data not shown).

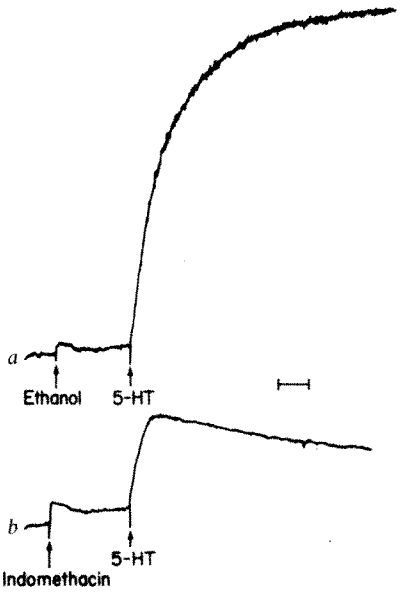


Fig. 4 Aggregation of platelets from CH cat no. 1 to 50 μM serotonin in the presence of indomethacin (20 μM) in absolute ethanol. *a*, Represents serotonin-induced aggregation 1 min after absolute ethanol treatment (20 μl ml^{-1} PRP). *b*, Represents serotonin-induced aggregation 1 min after indomethacin treatment. Scale bar represents 20 s. Similar aggregation was observed with aspirin (20 μM) in sodium acetate (data not shown).

normal and CH cat platelets to produce TXB_2 in a concentration-dependent fashion (Table 1).

It has been suggested that secretion precedes secondary aggregation, which is regarded as being due to increased extracellular levels of ADP and serotonin because PG endoperoxides and TXA_2 can induce a rapid release of serotonin and ADP from platelets[7,18]. This hypothesis is supported by the demonstration of an absence of secondary aggregation of platelets from most, but not all, patients with storage pool deficiency (a group of congenital disorders with a deficiency of platelet dense granules[19,20]). On the other hand, arachidonic acid, PGG_2 and PGH_2 cause aggregation of platelets from these patients[16] and of normal rabbit platelets depleted of dense granular constituents[15]. PGG_2 and PGH_2 can also cause aggregation of normal platelets without demonstrable nucleotide secretion[21]. These results suggest that secondary aggregation may be the result of a direct aggregating effect of TXA_2 and is independent of secretion. Indeed, secondary aggregation frequently precedes secretion and it has been suggested that secretion and secondary aggregation are parallel events which are not causally related[22].

It is clear that in the case of serotonin-induced aggregation of platelets from CH cats, secondary aggregation is not the consequence of increased extracellular levels to ADP and serotonin, but is directly related to the formation of TXA_2. The results, therefore, support the hypothesis of Charo and associates[21] that secondary platelet aggregation can occur independently of secretion. The reason that human platelets from some patients with storage pool deficiency have defective secondary aggregation is not clear. If these platelets are not deficient in cyclooxygenase or thromboxane synthetase, the lack of secondary aggregation could be explained by a positive feedback loop in which secreted substances are required to enhance the formation of TXA_2 to give complete secondary aggregation[3].

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Vanadate inhibits $(Na^+ + K^+)$ATPase by blocking a conformational change of the unphosphorylated form

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In the presence of extracellular K, intracellular orthovanadate is a potent inhibitor of $(Na^+ + K^+)$ATPase[1–3], and because this effect may have a physiological role[3–6], it is interesting to ask at which step in the enzyme cycle vanadate acts. In experiments with red cells and squid axons containing high concentrations of ATP, Beaugé and DiPolo[7,8] found that added vanadate modified the effects of extracellular K, or congeners of K, in a similar way to lowered ATP concentration. They suggested that vanadate might act by retarding the relatively slow, ATP-sensitive, conformational change that is thought to follow the hydrolysis of phosphoenzyme, when that hydrolysis is catalysed by extracellular K (ref. 9). There are several reasons for thinking that the same conformational change ($E_2K \rightarrow E_1K$) is the rate-limiting step in the conversion of E_2K to E_1Na that occurs when sufficient Na is added to $(Na^+ + K^+)$ATPase preparations suspended in low-K media in conditions which preclude phosphorylation[10–14]. The speed of the $E_2K \rightarrow E_1K$ change, in those circumstances, can be measured by the use of several different fluorescent techniques[10–12,15], and we report here experiments with a fluorescein-labelled enzyme to investigate the effects of vanadate. It turns out that, in the same conditions in which vanadate inhibits $(Na^+ + K^+)$ATPase activity, it greatly slows the rate of the conformational change $E_2K \rightarrow E_1K$.

Our method takes advantage of the increase in fluorescence that occurs when fluorescein-labelled enzyme changes from the form that exists in Na-free, K-containing media (E_2K), to the form that exists in high-Na media containing little or no K (E_1Na)[12,15]. The increase in fluorescence is thought to reflect a change in the immediate environment of the fluorescent groups brought about by the change in conformation of the enzyme.

Enzyme prepared from the outer medulla of pig kidney, by the method of Jørgensen[16], was labelled with fluorescein as described in Fig. 1 legend. (The procedure leads to the

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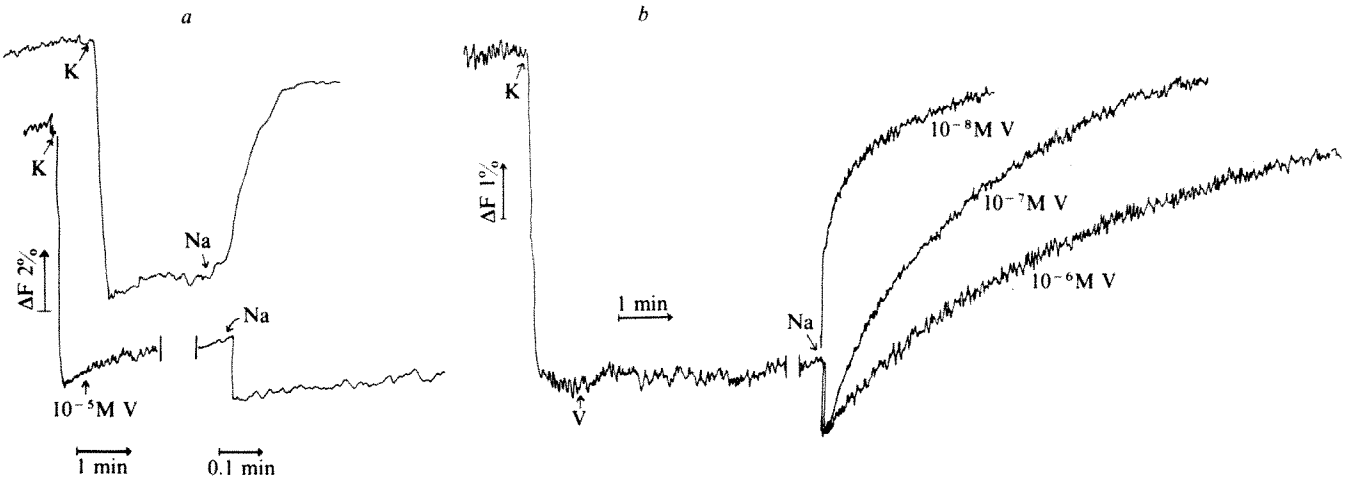
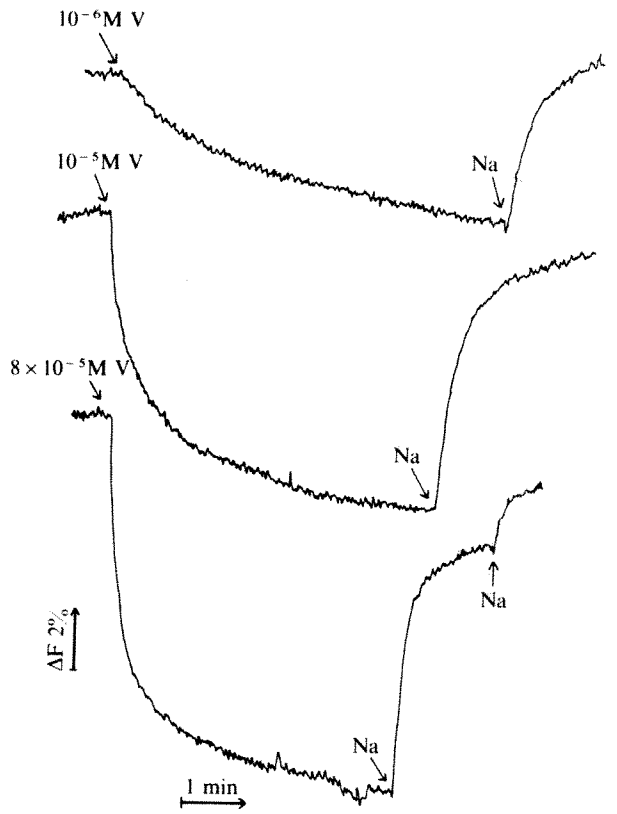


Fig. 1 Ten mg of enzyme (specific activity 20 μmol mg^{-1} min^{-1}) was incubated in 3.7 ml of a solution containing 100 mM NaCl, 50 mM Tris/Tris-Cl (*p*H 9.2 at 20 °C) and 5 mM 1,2-diaminocyclohexanetetraacetic acid. Fluorescein was added as a 150 μM solution in dried dimethyl formamide to give a final concentration of 7 μM. The suspension was allowed to stand for 30 min at 24 °C and was then dialysed at 0 °C against several changes of 150 mM Tris/Tris-Cl (*p*H 7.5 at 20 °C) containing bovine serum albumin (1 mg ml^{-1}) over a period of 48 h. For measurements of fluorescence, 60 μg of labelled enzyme was suspended in 2.5 ml of a solution containing 40 mM Tris/Tris-Cl (*p*H 8.0 at 20 °C) and 1 mM $MgCl_2$. Excitation was at 495 nm; emission was measured at 520 nm; the slit width was 10 nm, and the time constant of the machine was set at 0.3 s. The temperature was 20 ± 2 °C. *a*, Upper trace shows quenching of fluorescence by KCl (final concentration 16 mM) and the reversal of that quenching by NaCl (final concentration 80 mM). The lower trace shows that ammonium vanadate (10^{-5} M final concentration), added after the KCl, blocked the reversal by NaCl. Note (1) the gap in the lower trace represents a period of 5 min, designed to give the vanadate time to act; (2) the time scale was expanded 10-fold just before Na was added; (3) the apparent incompleteness of the reversal by NaCl is due to dilution of the enzyme; this also accounts for the sudden drop in fluorescence following the addition of NaCl in the lower trace. The drop due to dilution is not seen in the upper trace because it is masked by the rapid rise in fluorescence. *b*, Traces showing the effects of three different concentrations of vanadate on the rate of rise in fluorescence following the addition of NaCl. For 10^{-6} M vanadate, the complete curve is shown; for the other two vanadate concentrations only the part of the curves following the addition of NaCl is shown. Note (1) the gap in the trace represents a period of 5 min, designed to give the vanadate time to act; (2) the time scale was kept constant in these experiments; (3) with 10^{-8} M vanadate, the concentration of vanadate was much lower than the concentration of enzyme ($\sim 10^{-7}$ M), and the large amount of uninhibited enzyme accounts for the rapid initial rise in fluorescence, and the masking of the expected drop due to dilution.



incorporation of fluorescein groups into the large polypeptide chains of the enzyme, probably near the ATP binding sites. The labelled enzyme is unable to bind or to hydrolyse ATP, but it can still undergo the normal interconversion between E_1 and E_2 forms in response to changes in the concentrations of Na and K[12,15].) The fluorescein-labelled enzyme was suspended in a buffered medium, containing Mg but lacking both Na and K, in a fluorimeter cell fitted with a device that allowed additions to be made, and the suspension to be stirred, while the fluorescence was being recorded. In the Na-free, K-free medium, the enzyme was in the E_1 form and the fluorescence was high. When KCl was added to give a final concentration of 16 mM, there was a rapid fall in fluorescence as the enzyme was converted into the E_2K form (Fig. 1). (In the Mg-containing medium the K^+-induced fall in fluorescence is about 30% smaller than that observed in Mg-free media[15].) Vanadate was then added to give final concentrations in the range 0–10^{-5} M, and was allowed 5 min to act; no significant change in fluorescence occurred during this period. Finally, NaCl was added to give a final concentration of 80 mM. In the absence of vanadate, the fluorescence rose to its original level (allowing for dilution) within 5 s (Fig. 1*a*). With increasing concentrations of vanadate, the rise in fluorescence was progressively slower (Fig. 1*b*), until, with 10^{-5} M vanadate (Fig. 1*a*), the addition of Na had very little effect. The inhibition

Fig. 2 The reduction in the fluorescence of fluorescein-labelled enzyme by ammonium vanadate in the absence of KCl, and its reversal by NaCl (80 mM final concentration). The preparation of the enzyme, and the incubation conditions, were identical with those described in the legend to Fig. 1. Note (i) that the range of vanadate concentrations used in these experiments is higher than that in the experiments of Fig. 1; (ii) that ammonium ions were present in much too low a concentration to account for the fluorescence change; (iii) control experiments with Tris/Tris Cl showed that the effect of adding NaCl was not due to the increase in ionic strength.

by vanadate probably reflects the formation of a stable enzyme–K–Mg–vanadate complex[7].

In a second experiment, ammonium vanadate, in the concentration range 10^{-6} M–8×10^{-5} M, was added to enzyme suspended in the same Mg-Tris medium in the absence of both Na and K. As shown in Fig. 2, the addition of vanadate, in these conditions, itself caused a drop in fluorescence—faster at higher vanadate concentrations—which could be rapidly reversed by the addition of NaCl. The drop in fluorescence presumably reflected the net conversion of the enzyme to the E_2 form. This must have been caused by the vanadate ions, for, even at the highest concentration of ammonium vanadate, the ammonium ions alone could not have accounted for more than about 5% of the observed change. Furthermore, essentially similar results were obtained when Na vanadate was used. Addition of 80 mM NaCl before the addition of vanadate prevented the fluorescence change.

Inhibition of $(Na^+ + K)$ATPase by vanadate occurs only in the presence of Mg ions[1], which act at the intracellular surface[5,17]. It is therefore significant that the inhibition of the $E_2 \rightarrow E_1$ conformational change by vanadate could be detected only when Mg ions were present (not shown).

Taken together, the experiments reported here provide strong evidence that vanadate inhibits $(Na^+ + K^+)$ATPase activity by blocking the $E_2K \rightarrow E_1K$ conformational change that is thought to follow hydrolysis of the phosphoenzyme. When $(Na^+ + K^+)$ATPase preparations catalyse the hydrolysis of ATP in the absence of K, the rate limiting step is thought to be the hydrolytic step and not the subsequent conformational change. In the absence of K, therefore, we should expect the hydrolytic activity of the enzyme to be very much less sensitive to vanadate. Such a decrease in sensitivity is, in fact, observed, but the relation between K concentration and the effects of vanadate[7,8,18] cannot be wholly explained in this way.

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Purified cardiac cell membranes with high $(Na^+ + K^+)$ATPase activity contain significant NADH-vanadate reductase activity

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Vanadium compounds have been reported to inhibit the $(Na^+ + K^+)$ATPase activity of several cell membrane preparations[1–3], to have a positive inotropic effect on ventricular cardiac muscle[4], slightly increase myocardial cyclic AMP levels[5], stimulate fat cell adenylate cyclase[6], and cause natriuresis in rats[7]. Recently we have reported that vanadate produces a transient, initial stimulation of $(Na^+ + K^+)$ATPase activity in a cat heart cell membrane preparation, in addition to its well known inhibitory action on this enzyme system[8]. The transient stimulation occurred at the same concentration range as did the inotropic effects. There was, however, a serious discrepancy between the time courses of the two events. The former lasted only a few minutes, whereas the latter remained virtually stable for at least 30 min. The supposed stimulatory action of vanadate on $(Na^+ + K^+)$ATPase was deduced from an initial rapid loss of NADH in the ATPase assay used (optical coupled assay with an ATP regenerating system[9]). The oxidation of NADH was followed photometrically by continuous recording[8]. We now report that the rapid loss of NADH and hence the claimed transient stimulatory action of high concentrations of vanadate on the $(Na^+ + K^+)$ATPase activity is caused rather by an oxidation of NADH as a result of a NADH-dependent reduction of vanadate.

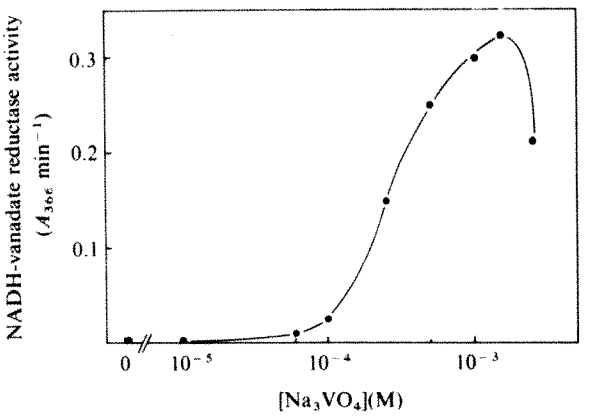


Fig. 1 Effect of vanadate on NADH-reductase activity in a calf ventricular cardiac cell membrane preparation. Calf cardiac cell membranes[10] (0.15 mg protein, 98% of total ATPase activity could be inhibited by 100 μm ouabain; $(Na^+ + K^+)$ATPase activity was 1.15 U per mg membrane protein) were incubated at 37 °C in 10 mM imidazole/HCl buffer, *p*H 7.25, 1.5 mM NADH (Boehringer Mannheim) and the stated concentrations of Na_3VO_4 (freshly prepared). Similar results were obtained with NH_4VO_3. Total volume 1.6 ml. The oxidation of NADH was followed photometrically at A_{366} by continuous recording. Similar results have been obtained with cell membranes from cat ventricles, human heart and human erythrocytes (not shown). Heating the cardiac cell membranes for 30 min at 60 °C completely destroyed enzyme activity. This curve exhibiting the oxidation of NADH (and reduction of vanadate) has the same concentration dependence as the formerly[8] reported rapid initial stimulation of NADH oxidation. This demonstrates that the coupled optical assay with NADH often used to measure ATPase activity is seriously influenced by NADH-vanadate reductase activity. The inhibition of enzyme activity at higher vanadate concentrations ($>10^{-3}$ M) might be due to the fact that NADH is oxidised very quickly.

The cardiac cell membrane preparation used in these and the former[8] experiments was isolated by the method of Pitts and Schwartz[10] but without the glycerol treatment. $(Na^+ + K^+)$ATPase activity was determined as 0.32 U per mg

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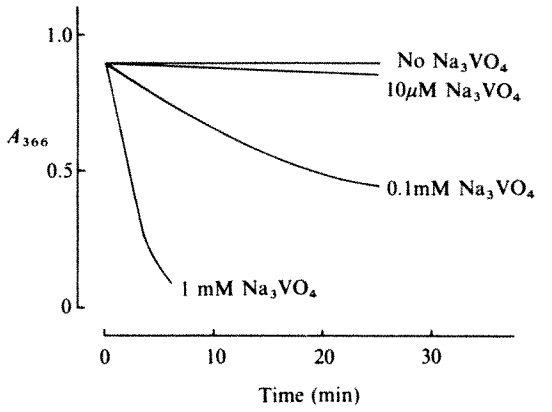


Fig. 2 Time and concentration dependence of NADH oxidation by NADH-vanadate reductase. Calf cardiac cell membranes[10] (0.02 mg protein) were incubated in 10 mM imidazole/HCl buffer, *p*H 6.75, 1.5 mH NADH and the indicated concentrations of Na_3VO_4; total volume 1.6 ml. The experiment was carried out in the same way as in Fig. 1. The curve shows that the oxidation of NADH is linear with time for several minutes.

membrane protein; 97% of total ATPase activity could be inhibited by ouabain (100 μM) and thus was defined as $(Na^+ + K^+)$ATPase. One enzyme unit is defined as 1 μmol ATP hydrolysed per min at 37 °C. Protein was measured as described by Lowry *et al.*[11].

When purified cardiac membranes[10] are incubated with NADH and different concentrations of vanadate but without ATP, a rapid oxidation of NADH, probably due to a reduction of $H_2VO_4^-$ (or $V_4O_{12}^{4-}$ or $V_3O_9^{3-}$)[12] to VO^{2+} is measured (Fig. 1). Thus, our previous interpretation of a stimulatory action of vanadate on $(Na^+ + K^+)$ATPase activity no longer seems justified.

A further analysis shows that the rate of NADH oxidation does depend on the concentration of vanadate (Fig. 2). Furthermore, increasing the amount of membrane protein in the test tubes results in an appropriately increased NADH oxidation (Fig. 3). As there is no NADH oxidation either in the presence of vanadate without membrane protein or in the presence of heat-denatured membranes, the isolated cell membranes must contain a NADH-vanadate reductase. The activity of this enzyme was stable for 4 d when kept at 4 °C and lost ~20% activity within 7 d (not shown). The vanadate concentration activating this enzyme half maximally (0.25 mM, Fig. 1) was rather high compared with the concentration of vanadium in serum or tissue (0.1–1 μM)[1,13]. Thus, unless higher vanadate concentrations at active sites are assumed, the enzyme does not

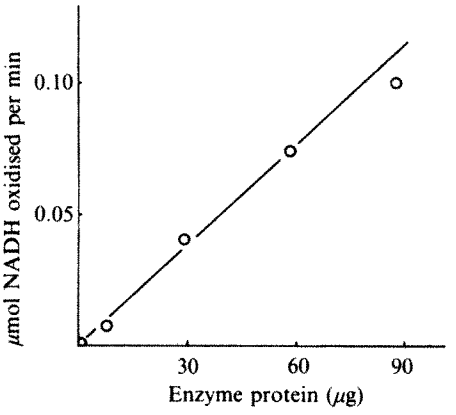


Fig. 3 The oxidation of NADH is increased linearly when the amount of enzyme protein is increased. The experiment was carried out as in Fig. 2 but with increasing amounts of calf cardiac cell membranes[10]. There was no NADH oxidation without enzyme.

function at optimal activity in physiological conditions. However, for most enzymes the Michaelis constant (K_m) has been found to be in this range (10–0.01 mH)[14].

As oxidation–reduction mechanisms are widespread in biological systems and have been found in high activities in the plasma membrane, cytoplasma and mitochondrial membrane of a variety of tissues, we think that this NADH-vanadate reductase activity is probably part of the well known plasma membrane NADH–oxidoreductase complex[15]. The exact significance of this enzyme for the action of vanadate at the molecular level requires further examination.

It is well known that vanadium compounds exhibit various valence states (II–V) and can be oxidised or reduced easily[15,16]. It is surprising, however, that purified cardiac cell membranes as used in these experiments contain such high amounts of vanadate reductase activity (about 1.1 U per mg protein). In the light of the results of Cantley *et al.*[1], suggesting that vanadate "may be an ideal specific regulator of $(Na^+ + K^+)$ATPase", this NADH-vanadate reductase in cardiac membranes might have physiological significance. Recently, it has been reported that most of the vanadate in red cells is reduced to vanadyl (VO^{2+}) and bound to haemoglobin[17].

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Alamethicin-induced single channel conductance fluctuations in biological membranes

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Alamethicin, a polypeptide of molecular weight ~2,000, induces voltage-dependent conductance phenomena in artificial lipid bilayer membranes that are in some ways similar to those found in excitable membranes[1]. Alamethicin-induced conductance is probably a consequence of the formation of predominantly cation-selective channels which span the bilayer, and single open channels fluctuate at random between several well defined conductance states[2–5]. Statistical analysis of single channel conductance fluctuations in planar lipid bilayers of various compositions has revealed that, whereas the conductance values are relatively constant in a variety of membranes, the average lifetime of the individual conductance states depends strongly on the lipid used to form the bilayer. This variability has tentatively been attributed to differences in lipid fluidity[6,7]. Alamethicin is therefore a probe of some aspects of membrane composition. The study of single alamethicin channels in various natural membranes would allow comparison with

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the well defined bilayer systems and might yield information on lipid properties of natural membranes that could be important for the functioning of natural membrane channels and receptors. The experiments reported here show that alamethicin channels have similar conductances in the sarcolemmal (surface) membrane of frog and rat muscle and in lipid bilayers formed from synthetic lecithin. The average lifetime of the conductance states is, however, one order of magnitude larger in the biological membranes.

Experiments were carried out on frog (*Rana temporaria*) cutaneous pectoris and rat (Sprague–Dawley) omohyoid muscles bathed in their respective Ringer solutions (for ionic composition see Figs 1 and 3 legends). Alamethicin was applied to a small patch of the extracellular surface of the sarcolemma from a pipette containing alamethicin at nominal concentrations of 9–50 $\mu g\ ml^{-1}$. Alamethicin-induced membrane currents were measured by the extracellular patch clamp method[8,9]. The membrane potential of the muscle fibre was controlled locally by means of a two-microelectrode voltage-clamp circuit.

Figure 1*a* shows membrane current fluctuations as a function of time, recorded from a patch of frog muscle membrane in the presence of alamethicin at a membrane potential of −110 mV. The tip of the pipette had been in contact with the muscle membrane for ~20 s during which the membrane remained in the resting state. At point A (Fig. 1*a*), an inward current step is recorded indicating a sudden spontaneous conductance increase of the membrane patch under investigation. The membrane current then fluctuates between six discrete levels of amplitude, some of which occur with a greater probability than others. At point B the current returns to the resting level. This type of current fluctuation closely resembles the pattern seen in lipid bilayer experiments in the presence of low alamethicin concentrations and has been ascribed to the formation of a single pore structure, undergoing transitions between different conductance states and then disappearing[4,5,10,11]. Current bursts like that shown in Fig. 1*a* occur at irregular intervals. The times between single bursts vary between a few seconds and about 1 min. It is difficult to investigate interval durations systematically because in most experiments after a few minutes, two or more alamethicin channels become active. This could be due to a continuous increase in alamethicin concentration at the membrane surface. It precludes single channel measurements from the same membrane patch over long periods.

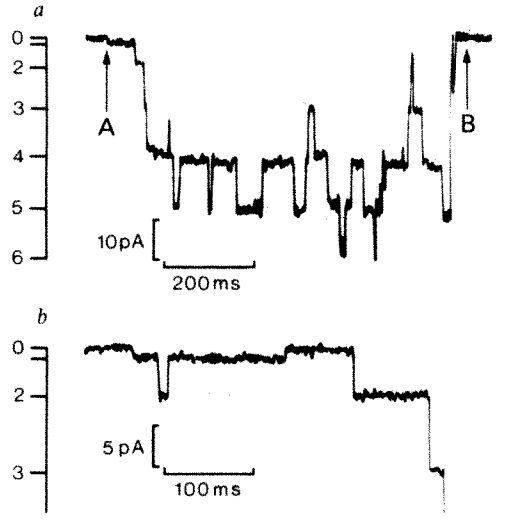


Fig. 1 Alamethicin-induced current fluctuations as a function of time. Frog sarcolemmal membrane at 18 °C. Inward current is downwards. The muscle was bathed in frog Ringer solution of the following composition (in mM): Na^+ 118; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121; HPO_4^{2-} 2.15; $H_2PO_4^-$ 0.5; *p*H 7.2. Alamethicin (9 $\mu g\ ml^{-1}$) dissolved in frog Ringer was applied to the extracellular side of the surface membrane through a patch-pipette with a tip diameter of approximately 2 μm. The pipette had a d.c. resistance of 2.5 MΩ. The seal resistances when the pipette was in contact with the muscle were 12–20 MΩ. This results in a slight underestimation (≤15%) of the true current amplitudes[9]. *a*, Oscilloscope traces of a current 'burst' at −110 mV membrane potential. At the time indicated by the arrow labelled A, an inward membrane current turns on, fluctuates for about 1 s between different amplitude levels and at the time indicated by the arrow labelled B, returns to the resting level. Six discrete levels of current amplitude are discernible. The different levels are indicated by the numbered scale on the left side. The level marked 0 corresponds to the resting membrane. *b*, Oscilloscope traces of the initial part of another current burst shown at a faster time base, and higher gain so as to illustrate more clearly the size of the first, second and third current levels. The membrane potential was clamped to −110 mV. The different levels are indicated by the numbered scale on the left side. Resting membrane corresponds to level 0. Both records were low pass filtered at 200 Hz with Bessel characteristics. For analysis of current step sizes and durations, records were stored on magnetic tape. They were replayed on a pen writer at reduced speed. Step sizes and durations were measured by hand and then analysed on a PDP 11/34 computer.

Table 1 Relative distribution probabilities, P_ν (%) and mean lifetimes, τ_ν (ms) of alamethicin conductance states in natural and synthetic membranes

Conductance state	Frog sarcolemma (−70 mV/18 °C)		Rat sarcolemma (−65 mV/20 °C)		Rat sarcolemma (−60 mV/30 °C)		Dioleyllecithin bilayer (−90 mV/18 °C)	
	P_ν	τ_ν	P_ν	τ_ν	P_ν	τ_ν	P_ν	τ_ν
1					39	50	9	1.5
2	10	40	74	130	27	35	20	2.8
3	26	35	19	70	16	35	37	2.4
4	41	40	5	45	12	30	21	2.7
5	19	25	2	40	5	25	6	2.5
6	3	20	—	—	1	10	—	—
7	1	20	—	—	—	—	—	—

In experiments on muscle fibres the pipette contained 9 $\mu g\ ml^{-1}$ alamethicin. Numbers in parentheses refer to membrane potential and temperature. The relative distribution probability was calculated by dividing the sum of durations of each current level by the total duration of the current bursts. The mean lifetime was obtained from the decay constant of the distribution of durations at each level fitted by a single exponential. P_ν and τ_ν values represent means obtained from at least 50 individual events obtained from bursts at the same membrane patch. The beginning of a single burst was defined by the appearance of a spontaneous current jump from the resting level as shown in Fig. 1*a*. The end of a burst was given either by the return of membrane current to the resting level or by the appearance of a second pore. At membrane potentials of −60 to −70 mV burst durations are longer than at higher membrane potentials and up to 1,500 amplitude transitions occur during a single burst. However, the resting and the first conductance states are not always unambiguously discernible at these potentials. We therefore had to start the statistical analysis with the second conductance level. In experiments with artificial membranes, synthetic dioleylphosphatidylcholine was used to form the bilayer according to the Montal–Mueller technique[17]. The ionic composition of the solutions in the two compartments on both sides of the bilayer was chosen so as to mimic the ionic composition of the extra- and intracellular fluid in frog muscle experiments. The two compartments contained the following ions (in mM): compartment *A*: Na^+ 119.8; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121.1; HEPES 4.8 (comparable to the extracellular bathing solution in muscle experiments). Compartment *B*: Na^+ 10.2; K^+ 140; Cl^- 2.2; HEPES 148 (comparable in its main cation composition to the intracellular composition of frog muscle[18]). HEPES was chosen to account for the large impermeable intracellular anions of muscle fibres. Compartment *B* was voltage clamped to −90 mV relative to compartment *A*. Alamethicin was added to compartment *A* at 0.5 $\mu g\ ml^{-1}$. The reversal potential for alamethicin-induced currents in this system was −38 mV, comparable to the experiments with frog muscle. The sequence of conductance states in this system is (in pS): $\Lambda_1 = 20$; $\Lambda_2 = 110$; $\Lambda_3 = 250$; $\Lambda_4 = 430$; $\Lambda_5 = 620$. (Recently, it has been found[19] that the lowest conductance state of the alamethicin pore in 1 M KCl at 21 °C is found at 19 pS. This would correspond to ~1.5 pS in the experimental conditions given in this study and is not resolvable by the present methods.)

Experiments with artificial bilayers have shown that the discrete current levels occurring during a burst are not integral multiples of a unit current step[2–4]. Figure 1*b* shows clearly that this is also the case in the muscle membrane, as the difference in amplitude between neighbouring current levels increases with the higher levels. Each current level is thought to reflect a discrete conductance state, ν, of the alamethicin channel. The different conductances, Λ_ν, of the channel in muscle sarcolemma were obtained by measuring current bursts at various membrane potentials and plotting the current–voltage relationship for the different states (Fig. 2). The relationship is approximately linear for all pore states in the range of membrane potentials measured. Extrapolation yields a reversal potential around −40 mV. The pore states vary over nearly two orders of magnitude in their conductance. In frog sarcolemma the Λ_ν values are in consecutive order (in pS): $\Lambda_1 = 20$, $\Lambda_2 = 100$, $\Lambda_3 = 260$, $\Lambda_4 = 460$, $\Lambda_5 = 660$, $\Lambda_6 = 860$. For comparison between channels in

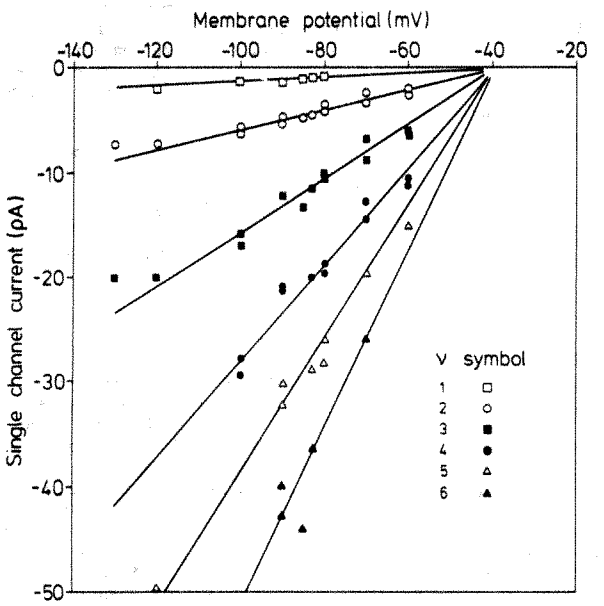


Fig. 2 Current–voltage relationships of the various conductance states, ν, of a single alamethicin channel in frog sarcolemma. The ordinate plots the average size of the inward membrane current during the individual conductance states (indicated by different symbols) as a function of membrane potential. Same experimental conditions as described in Fig. 1 legend with data pooled from two patches. The membrane potential was initially clamped to −130 mV until a current burst occurred. The potential was then shifted down stepwise to −60 mV. At each membrane potential the average size of the different discrete current levels occurring during a burst was determined from the amplitude distribution of the current burst. At each potential except at −60 mV, the level with the smallest average size was attributed to conductance state $\nu = 1$, the next larger level to state $\nu = 2$ and so on. At −60 mV membrane potential the average amplitude of the lowest level could not be measured accurately enough, therefore amplitude analysis was restricted to the higher states. The reversal potential of the alamethicin-mediated current obtained by linear extrapolation is near −40 mV. The conductance values, Λ_ν, of individual states ν obtained from the current–voltage relationships are (in pS): $\Lambda_1 = 20$; $\Lambda_2 = 100$; $\Lambda_3 = 260$; $\Lambda_4 = 460$; $\Lambda_5 = 660$, $\Lambda_6 = 860$. Similar experiments in rat muscle also gave a linear current-voltage relationship extrapolating to a reversal potential near −35 mV. The sequence of conductance values in rat (at 20 °C) is (in pS): $\Lambda_1 = 27$; $\Lambda_2 = 125$; $\Lambda_3 = 340$; $\Lambda_4 = 590$; $\Lambda_5 = 840$; $\Lambda_6 = 1{,}090$.

synthetic and muscle membrane, we have measured, in comparable experimental conditions (see Table 1 legend), alamethicin channel conductances in black lipid films made from synthetic lecithin. The Λ_ν values in this membrane are (in pS): $\Lambda_1 = 20$, $\Lambda_2 = 110$, $\Lambda_3 = 250$, $\Lambda_4 = 430$, $\Lambda_5 = 620$. The conductance sequences of alamethicin channels in the lipid bilayer and in muscle sarcolemma agree quite well. This similarity suggests that bursts like that shown in Fig. 1*a* are due to conductance fluctuations of a single alamethicin pore, as has previously been established in bilayer experiments[2–4].

The relative stability of the conductance states and the rates of transition between different states were evaluated by measuring relative distribution probabilities, P_ν, of each conductance state and their respective mean lifetimes, τ_ν, according to Boheim[4]. Table 1 shows the P_ν and τ_ν values of alamethicin pore states in frog muscle at 18 °C and −70 mV membrane potential. The channel adopts the three most probable states during ≈80% of the burst duration, the most stable state being the fourth one with a mean lifetime of 40 ms. For comparison, corresponding values of P_ν and τ_ν are given for the alamethicin channel in the lecithin bilayer. The distribution probabilities of the different pore states are comparable in both frog sarcolemma and lecithin bilayer, although the width of pore state distributions is somewhat narrower in the bilayer. However, there is a large difference in the average lifetimes of the pore states in the two membranes, the means of the τ_ν values of the three most probable states differing by more than one order of magnitude.

The difference in the open–close kinetics of the sodium channel[12] reported for frog and rat muscle prompted us to compare alamethicin channel state kinetics in both types of muscle. Figure 3*a* shows a recording of current through a patch of rat sarcolemmal membrane in the presence of a single conducting alamethicin pore. The conductances of corresponding pore states are somewhat larger in rat than in frog muscle, as would be expected from the different Ringer solutions (see Fig. 2 legend). The average lifetimes of the lower pore states in rat are much longer than in frog muscle (Table 1).

As indicated in Fig. 3*b*, pore state lifetimes are strongly temperature dependent. At 30 °C, the lifetimes of all conductance states are shorter than at 20 °C and are comparable to the values found in frog muscle at 18 °C (Table 1). The activation energies for channel closing were calculated from Arrhenius plots of the means of τ_ν values of the three most probable states. In frog (range 12–21 °C) the activation energy is ~40 kcal mol^{-1}, and in rat (range 15°–30 °C), ~20 kcal mol^{-1}. These values are larger by a factor of 4 and 2, respectively, than those reported for artificial membranes[11,13].

Thus, the above experiments show that alamethicin induces ion-conducting channels in muscle sarcolemmal membrane which show many properties that are qualitatively similar to those described for alamethicin channels in synthetic lipid bilayers. This suggests that the molecular mechanisms underlying channel formation are similar in both types of membrane. The main difference between the lecithin bilayer and the sarcolemmal membranes lies in the rates of transitions between the different conductance states. The major lipid components of sarcolemmal membrane are lecithins and cholesterol[14], and in lipid bilayer experiments, pore state lifetimes can increase with cholesterol content of the bilayer[6]. Therefore, the long lifetimes in sarcolemmal membrane may be due to the high cholesterol content. The lower rates of conductance state transitions in rat are in agreement with a similar (approximately two- to threefold) difference in sodium channel kinetics between frog and rat muscle fibres[12,15,16]. This could indicate that some difference between frog and rat sarcolemma, such as membrane viscosity, is responsible for the difference in channel kinetics. By measuring alamethicin channel kinetics in natural membranes in various conditions it might be possible to gain some insight into how membrane lipids can affect the kinetics of naturally occurring ionic channels.

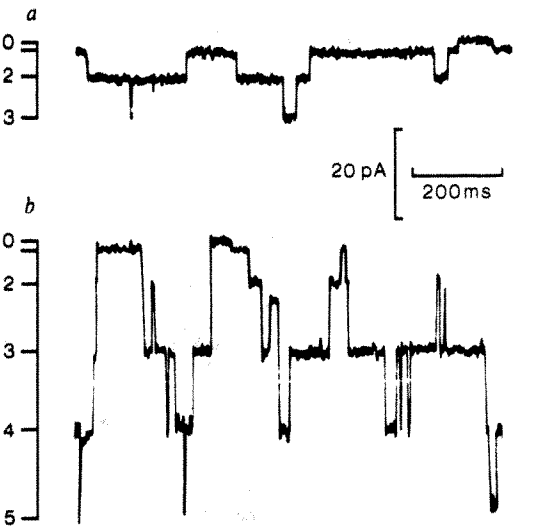


Fig. 3 Current fluctuations induced by alamethicin in rat sarcolemmal membrane at −95 mV membrane potential. Pipette contained 9 μg ml^{-1} alamethicin. Inward current is downwards. Muscle is bathed in rat Ringer solution of the following composition (in mM): Na^+ 156.4; K^+ 2.8; Ca^{2+} 1.0; Mg^{2+} 2.0; Cl^- 147.4; HPO_4^{2-} 8.2; $H_2PO_4^-$ 1.4; *p*H 7.2. *a*, Oscilloscope record of current fluctuations at 20 °C. Numbered scale on the left side indicates discrete current levels. Resting membrane level is indicated by 0. Note longer durations of individual current amplitude levels as compared with those observed in frog muscle at comparable temperature. *b*, Oscilloscope record of fluctuations at 30 °C. Calibrations are the same for both *a* and *b*.

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Endogenous viral genes are non-essential in the chicken

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DNA sequences homologous to the genomes of type C retroviruses are widespread among vertebrates[1]. Ten genetic loci containing endogenous viral DNA sequences have been documented in the white Leghorn chicken alone[2,3]. Six of these genetic loci are associated with the production of virus or of viral proteins in embryonic fibroblasts (refs 2–4, and S.M.A., L. B. Crittenden and E.G.B., in preparation) and one of the loci may be expressed in the erythroblasts of 5-day-old embryos[5]. The ubiquitous presence of endogenous viral genes among vertebrate species and the association of their expression with development of the haematopoietic system in the mouse[6] have led to the proposal that these genes are involved in ontogeny. In addition, the genes may be implicated in oncogenesis as in the case of the AKR mouse in which a high incidence of spontaneous leukaemia is associated with the expression of endogenous murine leukaemia virus genomes[7,8]. We report here the production of a fertile rooster which lacks avian leukosis virus-related endogenous viral genes and which seems to be completely normal and healthy. Thus, endogenous viral genes are apparently not essential for the normal development of the chicken. An endogenous virus-free state has also been reported for three species of jungle fowl[9] and for the *B*-type viral genes of the mouse[10].

The DNA of individual chickens was assayed for the presence of endogenous viral sequences by cleavage of embryo or erythrocyte DNA with restriction endonuclease *Sac*I, electrophoretic separation of the resulting fragments, transfer of the fragments, after denaturation, to nitrocellulose filters[11], and detection of viral sequences by hybridisation with ^{32}P-labelled 70S RAV-2 RNA. Endonuclease *Sac*I (an isoschizomer of *Sst*I (ref. 12)) was chosen because it gives a single major band for each site of residence of viral genes in the chicken genome[2]. Most chickens analysed were homozygous for an endogenous viral locus previously designated as *ev* 1 (ref. 2). However, during a mating experiment involving chickens of defined genotype, we identified two matings which gave rise to a small fraction of embryonic progeny in which no endogenous proviruses could be detected. Twenty-one progeny from these matings were hatched, and DNA was extracted from erythrocytes and assayed for viral sequences. The fragment patterns for the dams, sires and 21 progeny of the two matings are shown in Fig. 1. The bands corresponding to molecular weights of 5.8, 3.7 and 12×10^6 each represent an individual site of residence of endogenous viral genes and have been designated *ev* 1, *ev* 2 and *ev* 5 in accordance with previous nomenclature[2]. As can be seen in Fig. 1, the dams each carried *ev* 1 and *ev* 5 (lane *a*) whereas the sires carried *ev* 1 and *ev* 2 (lane *b*). Of the 21 progeny, 16 inherited *ev* 1, 12 inherited *ev* 2, and 11 inherited *ev* 5. One of the progeny (no. 15) apparently inherited no endogenous viral loci. These data are consistent with the sires being heterozygous for both *ev* 1 and *ev* 2 and the dams being heterozygous for both *ev* 1 and *ev* 5. In this case, half the progeny (on average) would be expected to inherit *ev* 2, half would inherit *ev* 5, three-quarters would inherit *ev* 1 and one-sixteenth would be expected to inherit no endogenous viral loci. These figures are very close to the actual frequencies of inheritance observed in the progeny.

To test further the DNA of rooster no. 15 for endogenous viral genes, radiolabelled DNA probes complementary to RAV-0 RNA, the *src* gene, and total cellular DNA were prepared and annealed to the DNAs of chicken 15 and chicken 17. As shown in Fig. 2, the probe to total cellular DNA, the *src* gene probe and the RAV-0 probe extensively to the DNA of chicken 17. The differences in the final percentage of each probe that anneals are most probably due to differences in the sizes of the cDNA probes (see Fig. 2 legend). The DNA of chicken 15 shows normal annealing to the cellular DNA probe and to the *src* gene probe, but shows little or no annealing to the RAV-0 probe. This result is consistent with the DNA of chicken 15 containing less than 2% of the sequences of RAV-0. The presence of *src*-related sequences in the absence of RAV-0 sequences is consistent with the observation that the endogenous *sarc* gene segregates independently of endogenous viral structural genes[13].

As a more sensitive test for the presence of RAV-0 sequences in the DNA of chicken 15, cDNA probes corresponding to the *gag*, *pol* and *env* genes of ALV and to the 5′ 101 nucleotides and the 3′ 600 nucleotides ('c' region) of RAV-0 were prepared and annealed to the DNAs of chicken 15 and chicken 17. As shown in Table 1, the DNA of chicken 17 anneals extensively to the five

Table 1 Per cent cDNA hybridised in chickens 15 and 17

Probe*	No. 15	No. 17
$cDNA_{5'}$	2	66
$cDNA_{gag}$	0	61
$cDNA_{pol}$	1	57
$cDNA_{env}$	1	51
$cDNA_{c}$	1	67

Hybridisation was carried out to a C_0t of 3.5×10^4 mol s l^{-1} as described in Fig. 2 legend. All values were corrected for a background of 1–2% with heterologous (salmon sperm) DNA.

* cDNA probes, corresponding to the *gag*, *pol* and *env* genes, were prepared from RAV-2 cDNA. Isolation and characterisation of these probes have been described previously[19]. ^{32}P-labelled $cDNA_{5'}$ (specific activity 4×10^8 c.p.m. per μg) contains sequences complementary to the 101 nucleotides at the 5′ terminus of RAV-0 RNA. The DNA was prepared as described by Haseltine *et al.*[20]. $cDNA_{5'}$ was purified on 10% acrylamide gels, first with the primer tRNA attached, and then after removal of the primer by alkaline hydrolysis. $cDNA_c$ was prepared from RAV-0 cDNA by two cycles of selection against short (9–11S) poly(A)-containing fragments of RAV-0 RNA[19], and thus corresponds to the 500–600 nucleotides adjacent to the poly(A) tract at the 3′ end of the RAV-0 genome.

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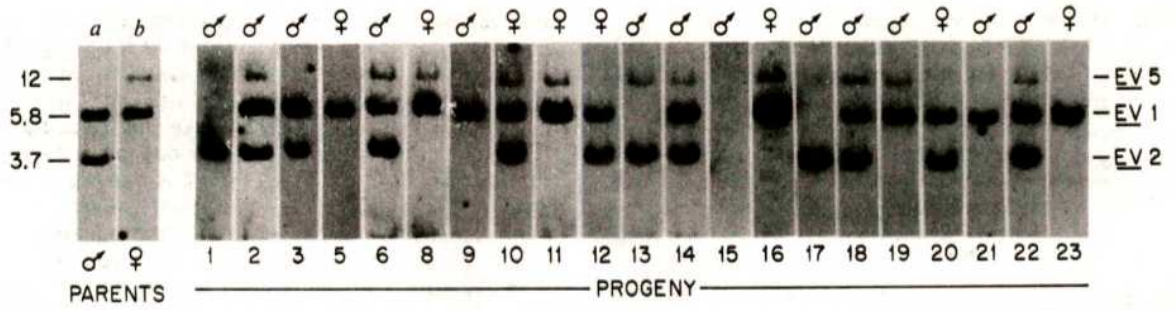


Fig. 1 *Sac*I digestion of chicken DNAs and identification of endogenous viral sequences. A marker of *Eco*RI digested λ ^{32}P-DNA was used to determine MWs, which are shown × 10^{-6}. Chicken DNAs were prepared[2] from erythrocytes and digested with *Sac*I. The resulting fragments were separated by electrophoresis on 1% agarose gels[2] and denatured and transferred to nitrocellulose filters by the method of Southern[11]. Viral sequences were detected by hybridisation with 70S ^{32}P-RAV-2 RNA as previously described[2]. The dams used in these matings were *gs*$^-$*chf*$^-$ hens from SPAFAS line 11. The sires used in the mating were F_1 progeny from a cross between a line 7_2 rooster and *gs*$^-$*chf*$^-$ hens from SPAFAS line 11.

probes, whereas the DNA of chicken 15 shows little or no annealing to any of the probes. From these results we conclude that chicken 15 definitely lacks avian leukosis virus-related endogenous viral genes.

We are now breeding rooster 15 to his female siblings and to the original dams to obtain stock to be used as the basis of a line of white Leghorns lacking endogenous viral genes. Twenty-one progeny have been hatched and are being tested for endogenous viral loci. One-quarter (on average) of the female siblings of rooster 15 should be homozygous for *ev* 1 because both the dam and sire of these chickens carried *ev* 1 in the heterozygous state. Matings with these homozygous females will produce no progeny lacking endogenous virus; the remainder of the matings should produce some of this type of progeny. Individuals lacking viral genes should be extremely useful in future studies on the possible involvement of these genes in ontogeny and oncogenesis. In addition, such chickens would provide a host for studying exogenous virus infection in the absence of endogenous proviral information.

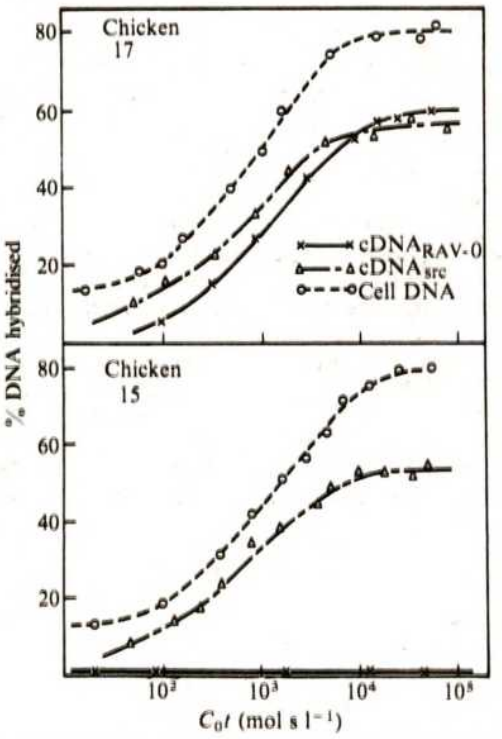


Fig. 2 Hybridisation of DNA of chicken no. 15 and chicken no. 17 to probes for total cell DNA, total RAV-0 sequences, and the cellular *sarc* genes (*src*). Probes were prepared as described by Hayward[19]. Re-association of cell DNA was monitored by addition of small amounts of ^{32}P-labelled cell DNA. Hybridisations were carried out in 4 × SSC using fragmented cell DNA at a concentration of 10 mg ml^{-1} and viral ^{3}H-labelled cDNA at 1 ng ml^{-1} (400 c.p.m. per reaction) in a total volume of 15–30 μl. Samples in sealed capillaries were heated to 100 °C for 3 min and incubated at 68 °C for various periods of time. Hybridisation was monitored by treatment with S_1 nuclease as described by Hayward and Hanafusa[21]. C_0t with values have been corrected to standard conditions of annealing as described by Britten and Kohne[22]. No corrections have been made for the differences in lengths of cell and probe DNAs. DNA lengths were approximately 150–200 nucleotides for unlabelled cell DNA, 150–200 nucleotides for ^{32}P-labelled cell DNA and 100–150 nucleotides for cDNAs.

The existence of an individual lacking endogenous viral genes implies that these genes are not essential in the chicken. How, then, does one account for their ubiquitous presence? The fact that domestic chickens lacking these genes have not been previously identified is probably a consequence of three aspects of the problem which we have observed in analyses of endogenous viral loci in over 500 individual chickens of more than 30 breeds (ref. 3 and J. Wyban, J. Fang and S.M.A., in preparation). First, there is a large multiplicity of loci in the total population of chickens; second, individual chickens usually possess several loci (J. Wyban, J. Fang and S.M.A., in preparation); third, the laboratory flocks of white Leghorns all contain locus *ev* 1 (ref. 2), an observation which may reflect the derivation of this breed from a limited gene pool. Given these three factors, the production, from random matings, of a chicken free of endogenous virus would be a rare event. If endogenous viral genes are indeed non-essential, why are they so prevalent in the population? It has been proposed that endogenous viral genes have arisen as a result of evolution of cellular genes[14]. Alternatively, they may have arisen as a consequence of retrovirus infection of germ-line cells[15]. The large multiplicity of endogenous viral loci observed in the chicken population and the heterogeneity observed among individual chickens are consistent with the theory that the viral loci arose through independent germ-line infections. Integration of the proviral genome at random or at any one of a large number of sites[16,17] would acccount for the observed large multiplicity of sites of residence of endogenous viral genes. In addition, differences in the genomes of the infecting virus and/or the occurrence of deletions, insertions or rearrangements in proviral DNA before integration would account for the heterogeneity observed among the endogenous viral genes themselves[18].

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Structural similarities between Thy-1 antigen from rat brain and immunoglobulin

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The molecular basis of cellular interactions is poorly understood but it is commonly believed that direct interactions between membrane-borne ligands and receptors are important. Membrane molecules whose expression is regulated in differentiation are candidates for these roles. Functional assays for cellular interactions are of such complexity that the traditional biochemical methods of purifying a molecule by following a functional assay are not applicable. It can be argued that the best approach is first to identify cell-surface molecules and then to attempt to identify functions on the basis of their molecular characteristics or by using the molecules or antibodies to them to perturb functional systems[1]. The Thy-1 (theta) antigen has been much studied because it is expressed in large amounts on some cell types but is absent from others. Thy-1 is probably the most abundant cell surface glycoprotein of rodent brain and thymus, yet its function is unknown (reviewed in ref. 2). On the basis of structural studies it was suggested that Thy-1 would be a candidate for displaying ligands for cell interactions and that the specific determinants involved might be carbohydrate[3]. We present here a preliminary report of further structural work dealing with the amino acid sequence and circular dichroism (CD) spectrum of rat brain Thy-1. The results suggest that this molecule has a structure resembling an Ig domain, which would not be consistent with an enzymatic function. The molecule probably exists to be recognised and the determinants involved could be protein or carbohydrate.

Thy-1 antigen has been purified from rat and mouse lymphoid cells[4,5] and brain[6–8]. In all cases the molecule is a glycoprotein with an apparent molecular weight of about 25,000 determined by SDS-polyacrylamide electrophoresis. The correct molecular weight as determined by sedimentation equilibrium is 17,500 for rat brain Thy-1 and 18,700 for thymocyte Thy-1 (ref. 9). In both cases the polypeptide has a molecular weight of 12,500, the rest of the molecule being carbohydrate[3,9].

Thy-1 glycoprotein seems to have a hydrophobic portion because it binds one micelle of deoxycholate and forms a regular oligomer in the absence of detergent[9]. Also the molecule is heavily labelled when mouse thymocyte membranes are affinity labelled with a photoactivatable reagent which partitions into the lipid bilayer (M. J. Owen and M. J. Crumpton, personal communication).

Extensive amino acid sequence data for rat brain Thy-1 have been obtained by sequencing the peptides isolated after tryptic cleavage of succinylated Thy-1 (D.G.C., A.F. W. and K.B.M.R., manuscript in preparation). Overlaps were defined by sequencing peptides cleaved at Glu residues by means of staphylococcal V8 protease. The sequence begins with pyroglutamic acid (PCA) and contains 3 Cys and 1 Met as shown in Fig. 1. There is a disulphide bond from Cys 85 to Cys 9 or Cys 19 because reduction is required to release the two peptides obtained after CNBr cleavage at Met 84 (ref. 9). The disulphide bond thus includes 75 or 65 amino acid residues and this is similar to the residue number within intra-chain disulphide bonds of immunoglobulin (Ig) domains (55 to 75 residues[10]).



Fig. 1 Cys and Met amino acids in the sequence of Thy-1 glycoprotein.

The sequence of 105 amino acids contains no obvious hydrophobic sequences of the type found in glycophorin[11] and the only extended stretch of amino acids without charged groups is from residue 3 to 15 (Fig. 3). Thus the mechanism by which Thy-1 interacts with the membrane remains unknown. It could be that a small hydrophobic peptide is missing from the C-terminus because Arg 105 has only been identified from a tryptic peptide. However, the known molecular weight and amino acid composition are quite consistent with the sequence already obtained, and it is thus unlikely that a large number of extra residues will be found.

When the Thy-1 sequence was compared with sequences of the domains of Eu myeloma IgG, homologies were apparent. These were particularly evident at the sequences around the Cys residues which form the intra-chain disulphide bond of the Ig domains. This is shown in Fig. 2, where sequences around

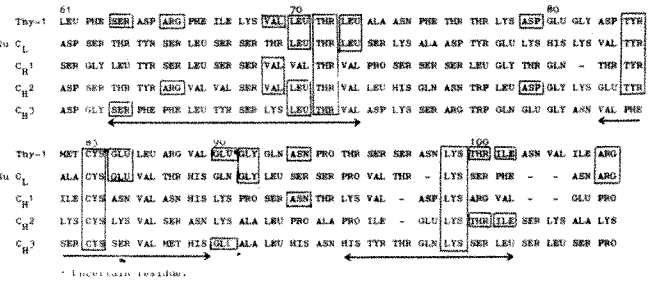


Fig. 2 Sequence comparisons between regions around Cys 85 of Thy-1 and the C-terminal Cys of the disulphide bridge in Ig domains. The sequences for the domains of Eu IgG are aligned as in ref. 12 for comparison of Ig domains with β_2-microglobulin. Thy-1 Cys 85 is aligned with Cys 81 in the β_2-microglobulin numbering. Positions are boxed where any Eu residue is identical to the appropriate Thy-1 residue. The arrows beneath the EuC_H3 sequences indicate the regions expected to be involved in β-sheet structures on the basis of the analysis of Beale and Feinstein[14].

the C-terminal Cys of the Ig domain are compared with those around Cys 85 of Thy-1. The alignment of the Ig domain is that used by Peterson *et al.*[12] for comparison of β_2-microglobulin with Ig and the Thy-1 sequence matches this alignment on both the N-terminal and C-terminal side of Thy-1 Cys 85 (the alignment with Thy-1 could be improved by altering the gaps in the Ig sequences in the region aligned with Thy-1 Lys 99). Most of the homologies occur where the Ig sequences are conserved, notably at residues in the regions aligned with Thr 71, Tyr 83 and Lys 99 of Thy-1. These sequences are in the regions which form the antiparallel β-sheet structure which is conserved in all Ig domains[13,14] (arrows in Fig. 2). In comparison with the Eu IgG domains the direct homology is best with the C_L and C_H2 domain sequences where identity with Thy-1 occurs at 9 and 10 positions respectively out of 42 and 44. For β_2-microglobulin compared over the same region as shown in Fig. 2 there is identity at 15 positions with C_L, 11 with C_H1 and C_H2 and 9 with C_H3 domain sequences[12].

A preliminary statistical analysis of the alignments shown in Fig. 2 has been done using the method of Moore and Goodman[15] which is based on the number of residues aligned and the minimum number of mutations needed to make the sequences identical. Given these values the probability that the alignment is due to a random occurrence can be read from a published table[15]. For the alignments shown in Fig. 2 the probability of a

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random relationship for Thy-1 versus respectively C_L; C_H1; C_H2 and C_H3 is: >0.01 <0.1%; 1%; >0.01 <0.1%; 1%. As controls for these values the Thy-1 sequence as in Fig. 2 was aligned with the N terminal Cys of the Eu IgG domains and in the four alignments the probability of a random association was >10%. In another set of controls the Thy-1 sequence as in Fig. 2 was aligned with C_H2 but with the Thy-1 sequences displaced forward or back 1 or 2 residues (2 residues would be more relevant because this would lead to alignment of structurally similar residues in regions of alternating hydrophobic amino acids in β-sheets[14]). Regardless of whether the misalignment was one or two residues the probability of a random association was always >10%. The scores for the alignments of Thy-1 with C_L and C_H2 are of the magnitude considered by Moore and Goodman[15] to indicate a high probability of an evolutionary relationship.

The sequences around the N-terminal Cys residues of Thy-1 were also compared with sequences around the N-terminal Cys of the Ig domains. In Fig. 3 it can be seen that there appears to be homology between the sequences around Thy-1 Cys 9 or Cys 19 and those around the N-terminal Cys of the Eu domains. It was also interesting to note that when Cys 9 and Cys 19 of Thy-1 were aligned (Fig. 3) four homologies were seen within the 13 residues C-terminal to each Cys. This may be a trivial observation but could indicate an internal duplication although this possibility is confused by the fact that Cys 19 is within the region of possible homology.

The size of the Thy-1 polypeptide, the disposition of the disulphide bond and the sequence homologies suggest that Thy-1 may have a structure similar to that of an Ig domain. This view is further supported by the fact that Thy-1 is highly resistant to proteolysis[2,3] as are Ig domains[10]. A more striking feature of Ig domains is their high content of antiparallel β-sheet structure and an absence of α-helical structure[13]. This gives rise to a characteristic spectrum for CD measured in the far UV region (190–230 nm). This spectrum shows a negative peak at 217 nm and an absence of the intense negative peaks at 208 and 222 nm which are characteristic of α-helix[16]. In Fig. 4 the CD spectrum for rat brain Thy-1 antigen is shown in comparison with a typical native Ig spectrum, namely, that of human myeloma IgD-Ha (ref. 17). Both spectra show the negative peak at 217 nm. The magnitude is greater for Thy-1 than for the IgD spectrum shown but is not markedly outside the range seen with different Ig molecules or their proteolytic fragments[16,17]. The Thy-1 spectrum shows no sign of intense optical activity at the wavelengths characteristic of α-helical structure. This further confirms the absence of a hydrophobic sequence like that in glycophorin which forms an α-helix which is readily detectable by CD[18].

Taken together these data present a reasonable case for suggesting that Thy-1 antigen has structural similarities to Ig domains. Further, it seems likely that Thy-1 and Ig share a distant evolutionary relationship, as is believed to be the case for β_2-microglobulin and Ig[19]. In comparing β_2-microglobulin and Thy-1 the similarities are the size of the polypeptides, the sequence homologies with Ig, the CD spectra and the cell surface location of the molecules. Differences are that β_2-microglobulin is found on most cell types in combination with the heavy chain of histocompatibility antigens and that it lacks carbohydrate[19]. Thy-1 has never been shown to associate with another polypeptide but appears to bind directly to the membrane. The functions of both Thy-1 and β_2-microglobulin are unknown, but it could be that these molecules exist to mediate cell–cell interactions and that a structure like the Ig domain is particularly suited to this role. The versatility of the domain structure is already proven by the large number of effector functions mediated by Ig constant region domains[14].

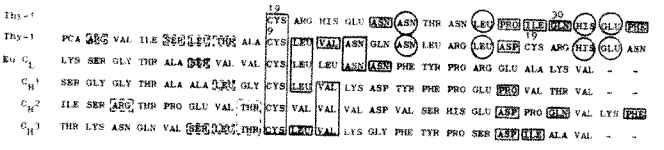


Fig. 3 Sequence comparisons between regions around the N-terminal Cys residues of Thy-1 and the N-terminal Cys of the disulphide bridge of Ig domains. Thy-1 sequences around Cys 9 and Cys 19 are aligned with sequences for the domains of Eu IgG aligned as in ref. 12 with Cys 9 or Cys 19 being aligned with Cys 25 in the β_2-microglobulin numbering. Residues are boxed where the residue of either Thy-1 sequence is the same as a residue in the Ig sequences. The residues boxed with dotted lines show homologies which would occur if a one residue gap was inserted in the Eu sequences at the N-terminal side of the Cys. The circled residues show homologies obtained on alignment of the sequences around Cys 9 and Cys 19 of Thy-1. At these internal homology regions there are no identical Eu Ig residues.

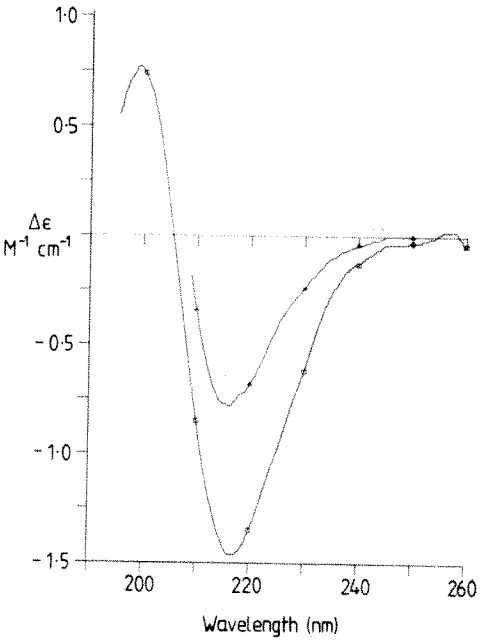


Fig. 4 CD spectrum of Thy-1 glycoprotein (○) and IgD(△). Thy-1 glycoprotein was purified from rat brains as previously described[6], with modifications involving the removal of lipid with chloroform:methanol extraction and the use of a monoclonal anti-Thy-1.1 antibody column for affinity chromatography (D.G.C., A.F.W. and K.B.M.R., in preparation). Deoxycholate was removed by ethanol precipitation of the glycoprotein[10] which was then solubilised in water. The CD spectrum was measured at 20 °C on a Jasco J41 CD spectrometer using a cell of 0.1-mm pathlength with glycoprotein at a concentration of 1.6 mg ml^{-1} (1.15 mg protein per ml). The CD spectrum of IgD-Ha[16] is shown for comparison. The CD spectra of brain Thy-1 in deoxycholate, or thymocyte Thy-1, were not distinguishable from that shown. For Thy-1 the mean residue ellipticity $[\theta_M]_{217} = 3{,}300 \times \Delta\varepsilon_{217} = -4.8 \times 10^3$ degrees cm^2 dmol^{-1}.

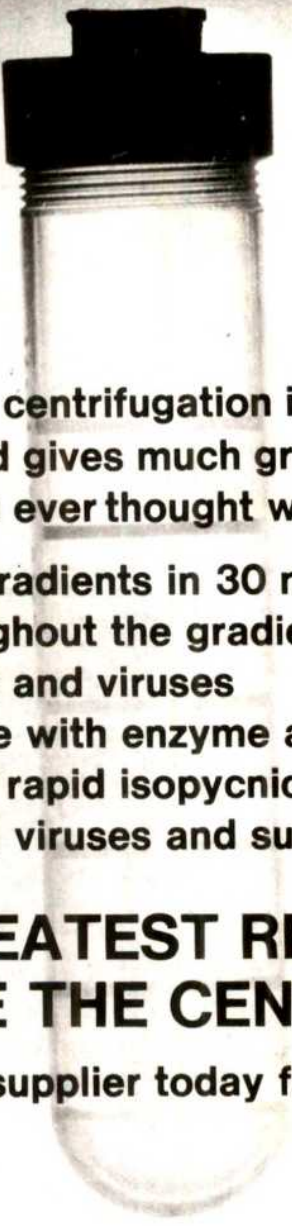
We thank Gaynor Newton, Tony Gascoyne and Dr C. A. Waller for technical assistance and Dr J. Gagnon for advice.

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reviews

Mediaeval scholarship

J. D. North

Science in the Middle Ages. Edited by D.C. Lindberg. Pp. 544. (Chicago University Press: Chicago and London, 1979.) £28; $40.

JULIET'S opinion notwithstanding, there is a great deal in a name, and "science" means something very different now from what it meant even a century ago (when, for instance, Lockyer chose to call his journal *Nature*). It is not necessary, on the other hand, to pay too much attention to those paradoxes who would have us believe that there was *no* science (in the current sense of the word) in the middle ages. Even putting aside Aristotelian and other natural philosophy ("great mother of the sciences", as Francis Bacon called it), there is an abundance of material in works on astronomy, optics, medicine, biology, technology, statics and the science of motion, which would be classed as essentially scientific by the modern scientist. The temptation to focus on this positive achievement of the middle ages has in the past been too great for many scholars. The pendulum has recently swung perhaps a little too far in the other direction, and we are growing accustomed to oh-so-scholarly articles uniting the most disparate of themes, so that no-one would now raise an eyebrow, for instance, at "The influence of judicial combat on the theory of refraction", or "Monastic poverty and the law of free fall". The most conspicuous merit of the collection of essays edited by Professor Lindberg is that their authors, despite an occasional attempt to tack, usually steer a sensible middle course between the two extremes.

There is little doubt that much scientific work of originality and worth remains hidden in manuscript, and that some is lost for ever. Of all the historian's main tasks, the one which tends to suffer least from these difficulties is that of tracing mediaeval influences in later periods. Many important studies have been opened up in this retrospective way; and here the study of mediaeval theories of motion, leading to Galileo and the successes of the seventeenth century, deserves pride of place. The chapters by William Wallace (on the philosophical setting) and John Murdoch and Edith Sylla (specifically on the science of motion) make excellent use of writings by such as Duhem, Maier and Clagett. The chapter by Murdoch and Sylla is notable for its mastery of the material, evidence of long and painstaking study. It is perhaps the best short introduction to the subject in any language. They stress the importance and pervasiveness of doctrines of motion to *all* mediaeval natural philosophy. Within their terms of reference, optics and statics were not considered as being strictly a part of natural philosophy, while astronomy is a difficult case, with one foot inside and the other outside the subjects. They discuss the mathematics of free fall, which managed to leave Aristotle on the sidelines, and the theory of impetus, which was hardly compatible with Aristotle; but they show how at every turn the books of Aristotle were, so to say, the intellectual driving force behind it all. The mathematical rules of the Mertonian (Oxford) and Parisian philosophers are remarkable enough, but moderately well known to scholars. What will perhaps come as a surprise to many readers is the importance they ascribe to the influence of the Church's doctrine of transubstantiation on the general theory of motion, so that "one of the two most prominent later mediaeval explanations of alteration, the so-called succession of forms theory, was originally taken seriously and worked out carefully as an explanation of the alteration and augmentation or diminution of the Eucharist". Another mediaeval tradition which might have been thought liable to lead to sterility but which is deemed influential, is that of the invention of sophisms within the context of logic. But all this activity was *secundum imaginationem:* it followed the creative imagination, and there was no question of any appeal to experiment.

The same was largely true of the science of optics, expertly discussed by the editor himself in a survey running from the ancient atomists to Descartes. Again the attack was on a broad front, making use of mathematical techniques (as of ray optics), but also taking in the physiology of the eye, the psychology of vision, and even theology. Here the key western texts were inspired not only by Aristotle, Euclid and Ptolemy, but by Islamic precursors such as Ibn al-Haitham and Avicenna. The editor is again himself responsible for a chapter on the transmission of Greek and Arabic learning. One can hardly complain about the brevity of the remarks on (for example) the work done in Castile under Alfonso X, as the chapter is so very full, tailing off only towards the end, where we might have had more about the *dissemination* of the translations done in Sicily, the Spanish peninsula, and elsewhere. The bibliographical references in this chapter are invaluable.

The mediaeval science of weights, discussed in a chapter by Joseph Brown, shared with optics and theories of motion an uncertainty of aim and content. Broadly speaking, two paths were followed: there was the quasi-dynamical approach of an early follower of Aristotle, and there was the Euclidean and Archimedean tradition. The mediaeval synthesis of the two was hesitant and imperfect. If we look to a later chapter by James Weisheipl on the "nature, scope, and classification of the sciences", I think we can detect one of the contributory causes, namely the sheer eclecticism of the mediaeval scholar. He wanted to combine an encyclopaedic coverage of his subject matter with that dangerous kind of confidence that comes from recording all conceivable points of view. In his more pragmatically orientated thinking, he was in many ways more successful than when he chopped logic and philosophy in the schools. (Divinity poses an unresolvable problem.) As John of Salisbury said somewhat impatiently, more time had been spent on Porphyry's questions than the House of Caesar spent in winning and in ruling the Empire of the World. Chapters on technology and economic progress (Brian Stock), science and magic, that is, a pragmatics of desire (Bert Hansen), astrology (touched upon by several authors in passing), and medicine (Charles Talbot), all show that when the chips were down the mediaeval scholar could dispense with the dialectical skills he had learned at his university — or at least that he could hold them in reserve to explain his failures.

Mathematics (Michael Mahoney), the science of matter (Robert Multhauf), and the universities as an institutional setting (Pearl Kibre and Nancy Siraisi), are all covered in ways which help the book to achieve a remarkable degree of comprehensiveness. Jerry Stannard's is a short but entertaining sketch of natural history, "a complex amalgam of fact and fancy". The two remaining chapters are by Edward Grant (cosmology) and Olaf Pedersen (astronomy), and are largely complementary. The first gives an outline of the Aristotelian cosmology with which the extraordinarily sophisticated picture arrived at by the mathematical astronomers had to be reconciled, if only for the sake of good form. The astronomy chapter provides an important reminder —

with many well chosen illustrations of instruments — that the astronomer at least was not averse to making observations of the real world. But to what extent he and other mediaeval scholars were doing real science is a question best answered by reading the collection as a whole. □

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Formal theory of ecosystems

Theoretical Systems Ecology. Edited by E. Halfon. Pp. 516. (Academic: New York and London, 1979.) £43; £27.95.

THE opening sentence of the preface to this book claims that systems ecology is having a large impact upon all aspects of environmental research, and that the systems approach, with its body of concepts and techniques, has broadened the ecologists' perspectives and attracted students into ecology from other disciplines. The preface goes on to suggest, however, that there has been a lack of communication between theoreticians and modellers and field ecologists, and that the purpose of this book is to try to bridge the gap in communication.

Is it true that systems ecology, or the application of systems theory to ecology, is having an impact on environmental research? Many ecologists would disagree with the statement, and there are as yet relatively few examples of practical action on environmental problems having been taken as a result of studies of systems ecology. There is certainly much understanding of the ways in which organisms respond to changes in their environment, and some of this modelling is based on concepts derived from studies of control systems. I suspect, however, that one of the reasons for the lack of communication between theoreticians and field ecologists is that the dependence of ecological models on systems theory is less than is believed by the theoreticians. Much of the modelling still based on statistical concepts developed even earlier than those of the systems analysts.

This volume contains 20 chapters written by 28 contributors, and divided into four main sections. Part I discusses some fundamental system problems, particularly the aggregation problem and its relation to sampling. Part II includes information on modelling approaches and philosophy, with emphasis on model structure and model properties. Part III introduces methodologies and computer techniques of system identification. Part IV contains studies on model analysis, principally of structural properties such as stability, flow analysis and general systems properties.

Undoubtedly, the book contains original contributions in theoretical systems ecology which may be important as a basis for future developments. As most of the chapters are almost unintelligible to anyone not already well versed in systems theory, however, the book does little, if anything to bridge the gap between theoreticians and practical ecologists. Indeed, until theoreticians can learn to state their conclusions in simple and unambiguous plain language, it is doubtful if systems ecology will really have the impact that systems analysts claim for it. At present, those of us who are attracted by this approach to ecology too often mistake our models for the real world, and are content to leave our description of these systems in the mathematical terms for which they were derived. Those who have to use our research find it difficult to translate from the mathematics to the plain statements of every-day usage.

One of the problems that need to be solved is terminology. The terms used in this book are claimed to be as uniform as possible, but different names are frequently used for the same concept or the same name for different concepts. The problem is said to be typical not only of systems ecology but also of general systems theory. This confusion seems to derive from the fact that the contributors follow the terminology of quite different theories of general systems, and the concepts of these theories do not often overlap. An early priority for theoreticians, therefore, would seem to be the resolution of these inconsistencies, and the development of a formal theory of ecosystems as a working definition for ecological research.

J.N.R. Jeffers

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Primary energy supply prospects

World Energy: Looking Ahead to 2020. Report by the Conservation Commission of the World Energy Conference. (IPC Science and Technology Press: Guildford, UK, 1978.) £12.50; $32.50.

THE objectives of the Conservation Commission were to evaluate, for the period 1985-2020, future primary energy supply prospects and to identify possible courses of action to overcome potential shortfalls in energy availability, particularly the impending decline of oil and gas supplies. The report is based on the work of a series of specialist study groups, dominated by OECD-centred experts.

The Commission notes that for industrialised nations there seems to be a decreasing requirement for additional energy supplies with increasing economic growth; by 2020, it seems that the energy coefficient might decline to one half its current average level in these countries (but it is unclear whether measurements are made in primary, delivered or final useful energy terms). In the case of developing countries, however, the incremental ratio of energy demand to economic growth will continue to increase.

Somewhat surprisingly, given the upsurge in public opposition to virtually any major energy siting decision (for example, refineries, coal mines and nuclear stations) and evidence of rising real capital costs, the Commission argues that "the energy supply which could be attained in the next few decades is easier to predict than is the demand for energy, because it is determined primarily by technology". The Commission forecasts that the share of conventional crude oil in total world primary energy supplies will decline from 40% at present to about 10% by 2020, with natural gas then contributing 20%, non-conventional petroleum (for example, tar sands, shales, geopressure zone gas) about 5%, coal 25%, and renewable sources 10%, with the balance, 30%, being provided by nuclear energy from fission.

Total primary energy demand is estimated to reach three times today's level of 9×10^9 metric tons of coal equivalent by 2020, involving major efforts in all energy resource areas. In particular, it implies tripling coal production, "doubling nuclear power plant capacity every six years" and substantial growth in solar energy.

The report envisages that about 30% of the energy that would otherwise have been needed by 2020 could be saved through technological improvements in conversion and utilisation. Further conservation, about 17%, is considered feasible through structural changes in the more mature economies, including saturation in car transportation and domestic energy demand and reduced utilisation by the energy-intensive industries.

The study is a valuable contribution to the literature, even though it omits sufficient discussion of issues such as public opposition, potential climatic impacts of increased energy consumption, geopolitical stresses likely to be prompted by international disparities in energy resource endowments, criteria for allocation of R and D expenditure, and more speculative and normative assessments of alternative world futures.

John Chesshire

John Chesshire is a Fellow of the Science Policy Research Unit, University of Sussex, Brighton, UK, and a member of the Unit's Energy Programme Group.

Speech Retarded and Deaf Children

Their Psychological Development

T. Fundudis, I. Kolvin and R. F. Garside

December 1979/January 1980, xviii+252pp., £15.80/$36.50 0.12.270150.X

In this book the authors describe a study in which outcome was investigated by examining a total population of speech delayed and of deaf children and an appropriate control group. Particular emphasis was paid to subsequent performance in speech and language development and behavioural functioning up to the age of eight. The authors also attempted to classify the disorders which share the symptom of speech delay. This is one of the few studies which have examined the later development of speech retarded and deaf children in depth and therefore contains much valuable and stimulating information, for psychologists, paediatricians, speech therapists, psychiatrists and neurologists.

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These two volumes, which contain papers presented at the International Conference on Psychology and Medicine held in Swansea in July 1979, provide the first full coverage of the work done in this important and growing field of behavioural medicine. The volumes present a considerable spread of research by psychologists, clinicians and others from hospitals and universities all over the world, with emphasis on the physical and social aspects of the relationship between psychology and medicine.

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European Monographs in Social Psychology No. 16

Jokes

Form, Content, Use and Function

Christopher P. Wilson

November 1979, x+252pp., £14.60/$34.00 0.12.758150.2

The author's aim is to provide a general portrait of humour, using data drawn from psychology, sociology and anthropology. He provides a detailed analysis of the defining features of jokes, their specific content, the formal categories into which they fall, the way form and content combine to evoke amusement, and the social and psychological reasons for, and effects of, joking. In particular, the author stresses the underlying unity of form as distinct from the superficial differences in the content of jokes. The book is notable for its attempt to draw together the divergent strands of research in this area and will provide a useful source of reference, as well as presenting a more coherent, overall theory of humour than is possible in a multi-author work. It is intended principally for psychologists, sociologists, linguists and anthropologists interested in the role of humour in their discipline. Much will also be found to interest students with a basic grounding in the social sciences.

Renal Function in Anaesthesia and Surgery

David R. Bevan

November 1979, xii+240pp., £15.20/$32.00

0.12.790581.1 (Academic Press) 0.8089.1160.0 (Grune & Stratton)

This book aims to review the effects of anaesthesia and surgery upon the normal kidney. It also deals with the problems of anaesthesia in the post-operative period, and provides guidelines to prevent further deterioration. To this end the text is divided into three sections: the basic principles of applied renal physiology; the effects of anaesthesia and surgery; and renal failure. It is hoped that application of physiological principles will enable the normal behaviour of the kidney in the post-operative period to be predicted, so that fluid and electrolyte therapy will follow a rational basis and any abnormality will be recognized earlier. This book is directed towards both clinician and researcher.

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Experimental Virology Series

Hepatitis Viruses of Man

Arie J. Zuckerman and Colin R. Howard

December 1979/January 1980, viii+270pp., £16.80/$39.00 0.12.083550.9

This second volume in the series deals with the hepatitis viruses of Man. The year 1968 saw the discovery of the association between Australian antigen and hepatitis type B: five years later hepatitis A virus was identified in faecal extracts. Evidence has recently emerged of a third form of hepatitis, which may be caused by more than one virus; also there are as yet unpublished reports on the insertion of hepatitis B virus DNA fragments into *Escherichia coli* plasmid with subsequent production of clones. This volume, which is not intended to be an encyclopaedic review of hepatitis virus, provides a comprehensive account of the more important published advances and exciting developments in the field of human viral hepatitis during the last decade.

Essentials of Postgraduate Psychiatry

edited by P. Hill, R. Murray and A. Thorley

November 1979, xviii+802pp., £21.50/$49.50

0.12.791983.X (Academic Press) 0.8089.1204.6 (Grune & Stratton)

The contributors, who were all until recently members of staff of the Maudsley Hospital, provide the trainee psychiatrist with a reasonably sized general text with a modern approach which contains all the information that is now essential for the competent practice of psychiatry. The contributors place great emphasis on research evidence rather than clinical wisdom, integrating the results of recent research into the main body of clinical knowledge. In the words of Dr Birley, Dean of the Institute of Psychiatry, writing in the foreword: "The result of their labours is a very readable volume which provides up-to-date reviews of most of the important fields of psychiatry, with a plentiful supply of references for further reading."

Social Insects

Volume 1

edited by Henry R. Hermann

September 1979, 456pp., £23.40/$36.00 0.12.342201.9

Subscription price: $31.50

This three-volume treatise on social insects is an endeavour to collate the works of modern researchers working in the field of insect sociobiology. The vastness of the subject insect sociality warrants at least the number of pages comprising these three volumes. In their totality, the inclusive chapters touch on all or almost all of the concepts studied to date. They discuss the major reports that have built our present store of knowledge on the subject; and they reveal gaps in our knowledge and lapses of continuity in our research and thinking. The contributors to these volumes have cumulated information not only on the work and theory already recorded in the literature but have often added new information and theory to what is already known.

Theoretical Systems Ecology

Advances and Case Studies

edited by Efraim Halfon

September 1979, 544pp., £27.95/$43.00 0.12.318750.8

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Advances in Inorganic Chemistry and Radiochemistry

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edited by J. J. Emeleus and A. G. Sharpe

December 1979, 480pp., £28.00/$48.00 0.12.023622.2

Chapter headings: H. D. B. Jenkins and K. F. Pratt: Lattice energies and thermochemistry of hexahalometallate (IV) complexes, A_2MX_6, which possess the antifluorite structure. **G. Stedman:** Reaction mechanisms of inorganic nitrogen compounds. **M. J. Atherton and J. H. Holloway:** Thio-, seleno-, and tellurohalides of the transition metals. **J. Mason:** Correlations in nuclear magnetic shielding, part II. **H. W. Roesky:** Cyclic sulfur-nitrogen compounds. **R. P. Burns and C. A. McAuliffe:** 1, 2-Dithiolene complexes of transition metals. **R. H. Prince:** Some aspects of the bioinorganic chemistry of zinc.

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edited by Peter Auer

April 1979, 374pp., £21.10/$32.50 0.12.014901.X

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Volume 2

December 1979, 288pp., £15.20/$26.00 0.12.014902.8

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Biochemistry and Physiology of Protozoa

Second Edition, Volume 1

edited by M. Levandowsky and S. H. Hutner, Consulting Editor: Dr. Luigi Provasoli

November/December 1979, 464pp., £26.20/$45.00 0.12.444601.9
Subscription price: $38.50

Chapter headings: M. Levandowsky and S. H. Hutner: Introduction. **J. D. Dodge:** The phytoflagellates: fine structure and phylogeny. **G. W. Pettigrew:** Structural features of protozoan cytochromes. **T. Goodwin:** Isoprenoid distribution and biosynthesis in flagellates. **E. Gantt:** Phycobiliproteins of Cryptophyceae. **A. D. Brown and L. J. Borowitzka:** Halotolerance of *Dunaliella*. **D. Klaveness and E. Paasche:** Physiology of Coccolithophorids. **F. L. Schuster:** Small amoebas and amoeboflagellates. **B. M. Sweeney:** The bioluminescence of dinoflagellates. **A. W. Coleman:** Sexuality in colonial green flagellates. **P. B. Applewhite:** Learning in protozoa. **H. Iwasaki:** Physiological ecology of red tide flagellates. **M. Levandowsky:** Mathematical models of red tides. **E. J. Schantz:** Poisonous dinoflagellates.

Second Edition, Volume 2

December 1979, 512pp., £20.85/$48.00 0.12.444602.7
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Chapter headings: M. Levandowsky and S. H. Hutner: Introduction. **D. Lloyd and M. H. Cantor:** Subcellular structure and function in acetate flagellates. **J. J. Wille, Jr.:** Biological rhythms in protozoa. **J. K. Kelleher and R. A. Bloodgood:** Microtubules. **J. M. Mato and T. M. Konijn:** Chemosensory transduction in *Dictyoselium discoideum*. **D. L. Cronkite:** The genetics of swimming and mating behavior in *Paramecium*. **Y. Nozawa and G. A. Thompson, Jr.:** Lipids and membrane organization in *Tetrahymena*. **J. R. Nilsson:** Phagotrophy in *Tetrahymena*. **G. S. Coleman:** Rumen ciliate protozoa. **B. M. Honigberg:** Biological and physiological factors affecting pathogenicity of trichomonads. **P. F. L. Boreham:** The pathogenesis of African and American trypanosomiasis.

Marine Mycology: The Higher Fungi

Jan Kohlmeyer and Erika Kohlmeyer

November 1979, 704pp., £38.65/$59.50 0.12.418350.6

An authoritative, up-to-date sourcebook on all aspects of the higher marine fungi, describing in detail all 209 filamentous species through the authors' own original investigations. The text includes 482 microphotographs and nine other illustrations, most of them never published before. Many of the species are depicted for the first time. The first, general part of the text deals with ecological groups and includes chapters on collection, culture, ontogeny, physiology, and phylogeny. The second, taxonomic-descriptive part contains illustrated keys, complete descriptions of each genus and species, together with substrates, geographical range, etymology of names, and literature. The authors advance a new theory positing two distinct groups of fungi in marine habitats.

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Protein phosphorylation

Protein Phosphorylation. By M. Weller. Pp. 557. (Academic/Pion: London, 1979.) £29.

ALTHOUGH proteins such as casein and phosvitin have been known for many years to contain covalently bound phosphorus, it is only since the discovery of enzyme regulation by phosphorylation-dephosphorylation in 1956 by Krebs and Fischer, and the discovery of cyclic AMP-dependent protein kinase in 1968 by Walsh, Perkins and Krebs that interest in protein phosphorylation has increased dramatically. The number of enzymes known to be regulated by phosphorylation-dephosphorylation has increased from 4 in 1969 to about 25 today, and the number of phosphoproteins (as opposed to enzymes) that have been identified exceeds 100. These include such familiar proteins as fibrinogen, ovalbumin and pepsin, in which the role of phosphorylation is still unknown.

The past five years have established protein phosphorylation as the major general mechanism by which intracellular events in mammalian tissues are controlled by external physiological stimuli and research in this area may well account for 5–10% of current biochemical and pharmacological research. Protein phosphorylation is not only implicated in enzyme regulation, but in the control of secretion, ion transport, protein turnover, chromosome condensation and viral transformation. Protein-bound phosphate also plays an important role in the binding of divalent cations such as iron and calcium, and in bone formation. Furthermore, many enzymes form phosphorylated reaction intermediates as part of their catalytic mechanism. Six different amino acid side-chains (serine, threonine, histidine, lysine, glutamic acid and aspartic acid) are known to be phosphorylated.

Dr Weller is to be congratulated on being the first person to attempt the monumental task of writing a comprehensive book on all these different aspects of protein phosphorylation. The book is readable, very comprehensive in its subject matter and remarkably free of gross factual errors and printing mistakes. It comprises 13 chapters and covers work published up to about the beginning of 1977. Nearly 3,600 references are listed at the end of the book.

As always the major problem of writing a book on a topic that is progressing rapidly, is that large sections are already out of date on the day of publication. Indeed, after reading this book one can see how much progress has taken place in some areas over the past two or three years. The chapters dealing with cyclic AMP- and cyclic GMP-dependent protein kinases and the molecular specificity of these enzymes is already well out of date. The sections on enzymes such as pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase and initiation factors of protein synthesis which merit only a few lines in the book, would have to be expanded considerably today. Unfortunately the calcium dependent regulator protein story, which has revolutionised our understanding of the role of calcium in cellular control, and the interrelationship between cyclic AMP and calcium ions, broke just too late to get included. Naturally, enzyme systems discovered since this book was written, such as those involved in triglyceride, steroid, neurotransmitter and hormone synthesis are not included. Perhaps the major criticism that could be levelled at the book is that it is too much a catalogue of facts, and more could have been done to relate protein phosphorylation to the integrated metabolism of the whole cell. On the other hand, chapters dealing with topics that are no longer such active research areas, such as the caseins and phosvitins, will remain complete and accurate accounts of work in these areas for many years.

However, there is no doubt in my mind that this book is a remarkable achievement and that it is an extremely valuable background reference book for all people engaged in research in this area.

Philip Cohen

Philip Cohen is Reader in Biochemistry at the University of Dundee, UK.

Sensory mechanisms of the spinal cord

Sensory Mechanisms of the Spinal Cord. By W.D. Willis and R.E. Coggeshall. Pp. 485. (Plenum: New York, 1978.) $35. Available in the UK, Africa and the Middle East from Wiley (Chichester, UK) at £20.

THIS book will bring some coherence and stability to a confused subject. The confusion is partly inherent in the subject itself: inevitably the details of physiological mechanism, and, for the most part, micro-anatomical structure, come from experiments on a variety of higher animals, and any relevance to sensory mechanisms in man has to be established with the greatest caution. This kind of experimental evidence has appeared explosively in the past 15 years or so and the degree of caution has left much to be desired. So, although this is a detailed and wide-ranging monograph, it is a pleasure to find that it begins with a cautionary list of the types of general theory which have been advanced at various stages to bridge this gap and points out where these have been flawed by experimental error, unjustified assumptions, or plain mental confusion. The various 'dual' theories about somatic sensation are examined in this way and all found wanting. And an elementary but important semantic point is made that the word "modality" in sensation is a subjective and not objective term — one which defines quality in what we feel, and not, as many continue to treat it to the great confusion of others, a term describing an instrumentally measurable attribute of a stimulus. Similarly the book ends with a serious attempt to define, where evidence allows it, the sense-organs, tracts and nuclei which form the various channels on which the brain depends for its information in different kinds (or modalities) of sensory experience.

The main bulk of the book is concerned with categorising and summarising recent evidence. Enough is said about sense-organs to form a sound basis for what follows; but the main detailed subject-matter is the anatomy and physiology of the sensory mechanisms of the spinal cord and medulla, that is, in general terms, the early stages of synaptic processing of incoming information and its channelling into a variety of new specialised paths. This is a field in which the authors and their close colleagues have a large personal experience and have made important contributions. The terminology and specialised techniques are sufficiently explained to make this quite easy reading. A good test of a comprehensive text like this is to use it. I used it to answer a variety of fairly esoteric questions to which I did not have a definitive answer and found its layout conducive to doing this quickly. The chapter titles and subheadings are reliable. The summaries are particularly helpful: each section has a short italicised summary and each chapter a set of concise conclusions. The bibliography is excellent.

The subject is advancing in full spate. This book is undoubtedly the best available text, indeed the only one of the present period, and it covers material published up to and including 1977. The form of the book is such as to allow updating and it is very much to be hoped that this will be done in due course. It is an expensive book for students, who would find the first and last chapters by themselves an excellent introduction to a subject which is potentially confusing and usually poorly dealt with in the general run of textbooks. The bulk of the book, however, is for reference or for the worker in the field. As such it is very much to be welcomed and not overpriced.

George Gordon

George Gordon is Reader in Sensory Physiology at the University of Oxford, and Research Fellow, Brasenose College, Oxford, UK.

Nature Vol. 282 15 November 1979

Assessment of pesticides

The Use and Significance of Pesticides in the Environment. By F. McEwen and G. Stephenson. Pp.538. (Wiley: New York and Chichester, UK, 1979.) £16.75.

THIS book is based on lectures for a course that the authors have run for several years at the University of Guelph, and it is intended to give senior undergraduates a broad assessment of pesticides. The authors have achieved a high degree of detached assessment, based on the premise that although pesticides are virtually essential they also can and do present serious problems. On occasion this detachment almost becomes a fault, as both sides of some issues are presented in such a way that the authors' opinion is not immediately obvious. Students are unlikely to have the background of knowledge and experience to decide for themselves what reasonable assumptions can be drawn from a series of disjointed facts unless there is a fairly clear critical assessment of the issues involved.

The introductory chapter outlines the development of modern synthetic insecticides during and since the Second World War, the consequent problems of resistance to insecticides, the contamination of the environment by persistent insecticides, the effects on wildlife, possible hazards to human beings, and the possible role of integrated control. Several brief chapters then describe the types of issue raised by the use of pesticides, their use in public health, agriculture, forestry and 'rights-of-way', and for domestic purposes. No mention is made of industrial uses, particularly the treatment of wool and timber, which is causing much discussion in some parts of the world. A brief elementary but good chapter describes current testing procedures for the development of new pesticides: it should perhaps have referred to the current pressure for increasingly extensive and expensive toxicity tests, with the possible repercussions on the ability and willingness of chemical companies to develop new pesticides.

The major part of the book falls into two sections. The first gives brief accounts of the chemical, physical and biological properties of many individual pesticides, and the second describes the occurrence, movement and effects of pesticides in various parts of the environment — soil, water, air, food, birds, mammals and the biosphere.

This is a book that requires intelligent skimming, which would be helped if each chapter started with a list of its subdivisions. There is much material that may be useful for reference, but surely not all to be considered in detail. Examples are taken principally from North America, but this does not altogether detract from the book's value for a British or European readership. For instance, the accounts of spraying against spruce budworm in the forests of New Brunswick, the consequent effects on salmon in the Miramichi River, and the effects of parathion on agricultural workers in California are all of general interest.

I would fault the book on details. The section that describes individual pesticides, despite its extensive coverage, is not too well integrated with the section on pesticides in the environment: residues of dichlobenil and BHC are mentioned, but the student will find no account of their chemistry and properties. The decline of peregrine falcons (*Falco peregrinus*) in Great Britain is attributed to eggshell thinning, instead of the acute toxicity of dieldrin poisoning. The authors do state that pesticides in water can be associated with particulate matter, but quote LC_{50} values for DDT of up to 2,400 p.p.10^9, whereas the best estimates for the maximum solubility of DDT in water are a little over 1 p.p. 10^9. Similarly no comment is made on this aspect of Wurster's work on the effects of DDT on phytoplankton at concentrations of 10–100 p.p. 10^9.

On occasion too one would like the authors' opinions. The Delaney amendment in the USA, which prohibits the use on food of any pesticide that has been shown to be carcinogenic, is mentioned without any comment, although eighty pages later two and a half pages are devoted to testing for carcinogens. Again we are told, without comment, that "environmentalists" have criticised severely the use of dieldrin against fire ants. This is a programme of which those outside of North America may have heard but may lack detailed knowledge.

There is remarkably little about resistance — it is not listed in the index at all — nor about the wider ecological implications of pesticide use. To conclude, this is a book worth dipping into, although it will not suffice on its own for a broad assessment of pesticides. **F. Moriarty**

F. Moriarty is a Principal Scientific Officer at Monks Wood Experimental Station, Institute of Terrestrial Ecology, Abbots Ripton, UK.

Crystal structure determinations

Inorganic Molecular Dissymmetry. By Y. Saito. Pp. 167. (Springer: Berlin, Heidelberg and New York, 1979.) $42.90; DM78.

PROFESSOR Yoshihiko Saito pioneered in 1955 the determination of the absolute stereochemical configuration of chiral transition metal coordination compounds by the anomalous X-ray scattering method, following the application of the technique by Bijvoet in 1951 to (+)-tartaric acid. The latter study showed that the arbitrary stereochemical convention of Emil Fischer, proposed in 1891, is in fact correct, while the former demonstrated that Werner's prototype dissymmetric complex, (+)-tris(ethylenediamine)cobalt(III),(+)-$[Co(en)_3]^{3+}$, has the morphology of a left-handed three-bladed propeller, later termed the *Λ*-configuration.

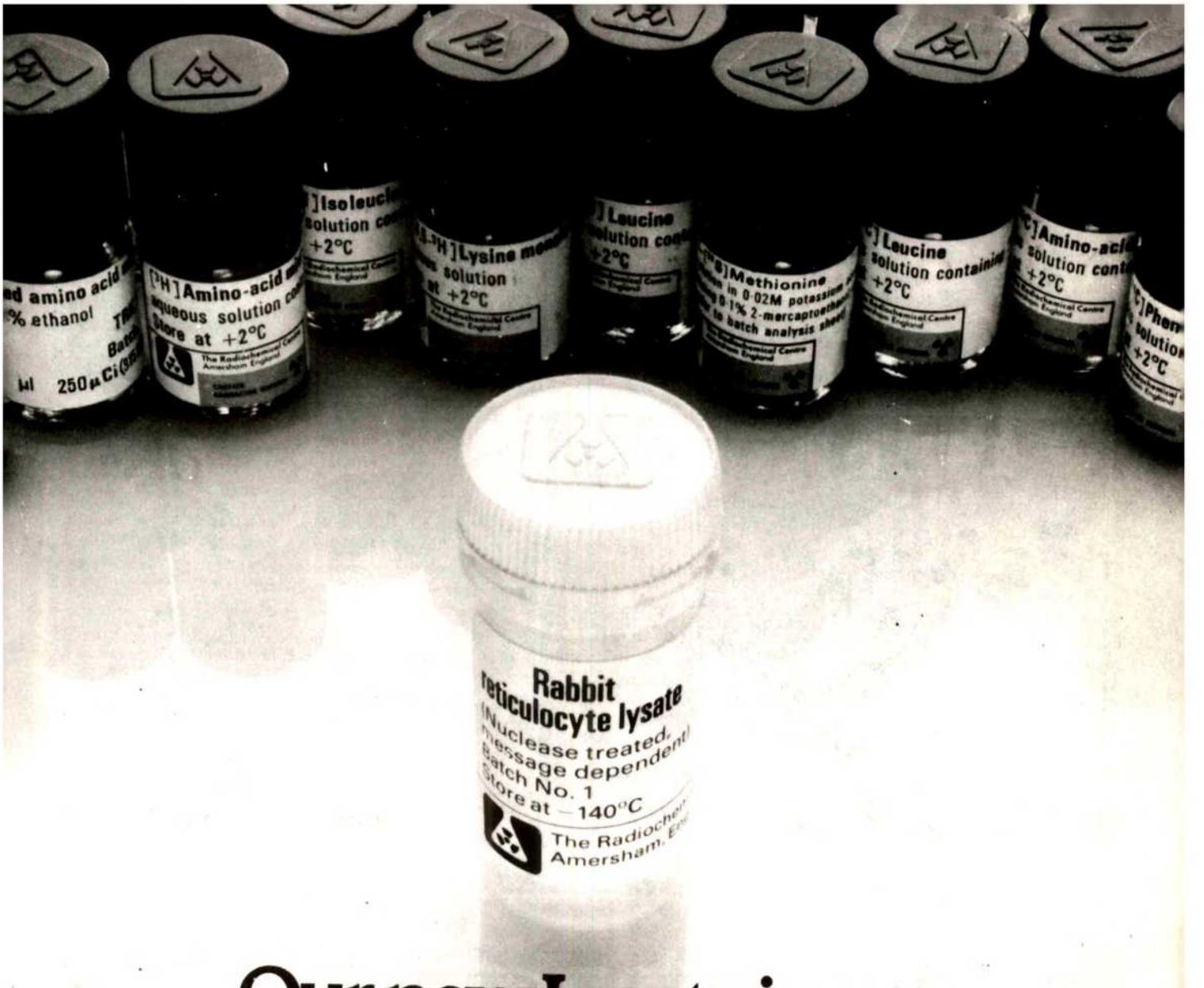
More recently Professor Saito has measured the electron-density distribution around the metal ion in transition metal complexes from the difference synthesis maps obtained through the use of automated X-ray diffractometers. In the case of $[Co(en)_3]^{3+}$, for example, it is found that the cobalt(III) ion is almost uncharged, in agreement with Pauling's electroneutrality principle, and that the 3d-electron density distribution is aspheric, with lobes located at the corners of a cube centred on the metal ion, while the electron density of the nitrogen non-bonding electron-pairs forming the coordinate bonds are somewhat displaced from the line joining the nitrogen atom to the metal ion.

His book, *Inorganic Molecular Dissymmetry*, provides a concise account of the principles underlying these studies and of their application in selected crystal-structure determinations, together with the theoretical basis and the practical articulation of conformational analysis applied to metal chelates, and of the investigation of d-electron optical activity in chiral transition metal complexes. The introduction to the book covers the types of isomerism encountered in metal complexes, and used by Werner in his structure theory, symmetry notation, and the specification of absolute configuration. The illustrations are clear and instructive, as are the mathematical appendices.

The book is directed towards a general and synthetic understanding of chiral molecules, and their unique property of optical activity, in the field of transition metal chemistry. The level of treatment is suited to graduate or advanced undergraduate teaching. For these roles, and for library reference, the book is strongly recommended.

S.F. Mason

S.F. Mason is Professor of Chemistry at King's College, University of London, UK.



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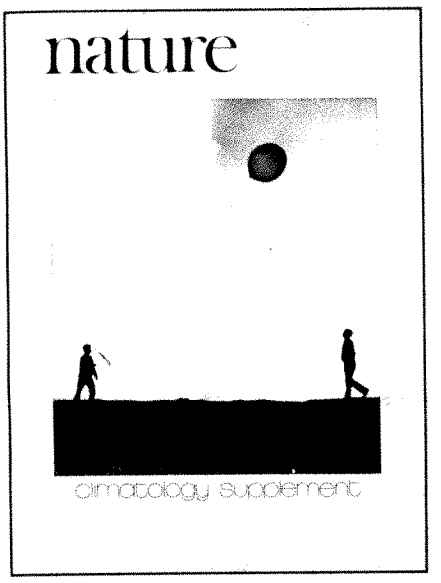
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Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 292. No. 1390: Ultrasonic and Radiological Methods in Engineering and Medical Diagnosis: Limitations and Future Prospects. A Discussion organized by Sir James Menter, F.R.S., G.N. Hounsfield, F.R.S., and J.G. Young. Pp. 135-305 + 13 plates. UK £14.90; Overseas £15.30. No. 1391: The Shape of a Pendent Liquid Drop. By P. Concus and R. Finn. Pp. 307-340. UK £2.50; Overseas £2.80. No. 1392: Ninlinear Effects Upon Waves Near Caustics. By D.H. Peregrine and R. Smith. Finite-Amplitude Deep-Water Waves on Currents. By D.H. Peregrine and G.P. Thomas. Pp. 341-390. UK £3.65; Overseas £3.95. B: Biological Sciences. Vol. 287, No. 1018: The Evolution of Bird Coloration. By R.R. Baker and G.A. Parker. Pp. 63-130. UK £4.95; Overseas £5.25. (London: The Royal Society, 1979.) (39

Science Research Council. Making the Most of Materials: Materials and Energy Conservation in the Materials Processing Industries. Pp. 183. (Swindon, Wilts.: The Science Research Council, 1979.) (39

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APPOINTMENTS VACANT

THE UNIVERSITY OF MANCHESTER
Oxford Road, M13 9PL
DEPARTMENT OF ZOOLOGY

TECHNICIAN (Grade 5)

or the Genetics section to assist with esearch on mosquitoes and other tropical nsects. Applicants should have a degree, HNC or equivalent and several years' elevant experience.

Salary on the scale £3,770 to £4,320 pa under review).

Applications together with the names of wo referees, should be sent to Dr R J Wood, Department of Zoology. 3734(A)

UNIVERSITY OF MANCHESTER

LECTURESHIP IN PHYSICS

pplications are invited for the above post. he Lecturer will be expected to take part in aching in the Physics Department and to rry out research on Experimental lementary Particle Physics, in either the ADE Collaboration on the PETRA e+e- orage ring at Hamburg or the Photon llaboration on the OMEGA detector at e SPS at CERN, Geneva. Further formation can be obtained from ofessor P G Murphy. Salary within the nge £4,333 to £8,992. Superannuation. tial salary will depend on age and perience but is unlikely to be above ,199.

Applicants with appropriate experience experimental particle physics should ply to the Registrar, The University, anchester M13 9PL, from whom further rticulars and application forms are tainable. Completed forms should be urned by December 15th. Quote ref: 4/79/N. 3730(A)

ROTHAMSTED XPERIMENTAL STATION
Harpenden, Herts AL5 2JQ

ADDITIONAL ORGANIC CHEMIST

h good practical ability required to work eam synthesizing powerful insecticides ted to the pyrethrins. Excellent instru- ntal facilities available. This temporary t (2 years) is provided by the National earch Development Corporation to loit promising previous work. The ointment should start as soon as sible. Minimum qualifications: HNC, s degree or equivalent, with five years' vant post-qualifying experience.

ppointment in grade of Higher ntific Officer, £4727 to £6211 (£5097 to 37 from 1.1.80). Starting salary accord- to qualifications and experience. Non tributory superannuation.

pply in writing to the Secretary giving es and addresses of two referees and ting Ref 415 by 30th November, 1979. her particulars on request. 3757(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

POST-DOCTORAL POSITION
Cambridge

For either an experienced electron microscopist or a biophysicist with interests in laser and fluorescence work. From 1st January 1980 for 3 years with a mammalian experimental embryology group. Applications to Dr M Johnson, Department of Anatomy, Downing Street, Cambridge. 3758(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL
(University of London)
Denmark Hill,
London SE5 8RX

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER 'B'

required for the Chest Unit in this Medical School. Work will involve contact with patients and assisting doctors with research into diseases of the lung.

Minimum requirements, 5 'O' levels, 4 in Science subjects including maths, day release available. Post suitable for school leaver.

Salary according to age, qualifications and experience.

Applications, in writing, giving full details should be sent to the Secretary of the Medical School at the above address. Closing date 29th November, 1979. 3732(A)

SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING
PRINCIPAL SCIENTIFIC OFFICER

A vacancy exists in the staff of the Potato Breeding Department at the Scottish Plant Breeding Station for a Potato Breeder in the grade of Principal Scientific Officer.

The person appointed to fill this post will be responsible to the Head of Department for a wide range of potato breeding and associated work carried out by the Department, and will be expected to collaborate with other senior staff members in the up-dating and development of the department's breeding programme. The appointee will be required to lead teams engaged in field work, which will involve travel to trial sites in various parts of the United Kingdom, and to liaise with collaborators in the potato processing industry and in other breeding organisations.

Applicants should be experienced plant breeders with a flair for planning, analysis and interpretation of field trials data, and a facility for collaboration.

Qualifications: 1st or upper 2nd class Honours degree in a biological subject, with considerable relevant post-graduate research experience.

Salary: In the range £7,534 to £9,646 per annum.
The post is superannuable.

Application forms may be obtained from The Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF, to whom completed forms should be returned not later than 7th December 1979. 3727(A)

SYDNEY HOSPITAL

Director of Medical Research

Kanematsu Memorial Institute

Applications are invited from medical graduates with senior degrees and substantial research experience for the position of Director of Medical Research.

Sydney Hospital, the oldest Hospital in Australia, was founded 191 years ago. It is situated in the centre of the City of Sydney and is a Teaching Hospital of the University of Sydney. During the year 1978/79, 18,828 inpatients were admitted to its 476 beds and 250,000 persons attended as outpatients.

The Kanematsu Memorial Institute, an integral part of Sydney Hospital, was established in 1933 and provides a clinical service as well as both clinical and applied research.

The Director will be in charge of the Medical Research Department which has well equipped laboratories, a workshop, an animal house and its own associated wards. The Director may develop fundamental and/or clinical research along any line appropriate to the work of the Hospital.

The salary will be that of a Senior Medical Specialist (at present up to A$39,061). There is also an administrative allowance and a right of limited private practice.

A supporting statement giving information about the Department, the Institute, and the Hospital, as well as details of the conditions of employment and instructions to applicants is available from the undersigned.

Applications close on 1st January, 1980, and should be forwarded to the undersigned in an envelope marked "Director of Medical Research". Further enquiries should be addressed to Chief Executive Officer, Sydney Hospital, Macquarie Street, Sydney, NSW 2000, Australia. 3723(A)

Entomologist

Fisons Limited, Agrochemical Division is a major UK producer of chemicals for agriculture, public health and industrial applications.

The Research and Development Centre for these rapidly growing operations is based at Chesterford Park, a renovated country house set in its own gardens near Saffron Walden, Essex, providing an attractive location for a secure career.

We are currently looking for an Entomologist (male or female) to join a team of scientists engaged in assessing the value of pesticides as new compounds, new formulations or new uses, under laboratory, greenhouse or field conditions both in the UK and abroad.

You should have a post graduate qualification in applied entomology, but graduates with a good honours degree in a related subject and experience should not be deterred from applying.

We offer a competitive salary supported by a substantial range of fringe benefits normally associated with a major industrial company including assistance with relocation expenses where appropriate.

For full details and an application form please write to or telephone: Peter England, Personnel Officer, Fisons Limited,
Agrochemical Division,
Chesterford Park Research Station,
Saffron Walden, Essex CB10 1XL.
Telephone: Saffron Walden 23542.

FISONS

3772(A)

REGIONAL NMR FACILITY

The University of Illinois has recently been designated as an NSF Regional Instrumentation Facility for NMR Spectroscopy. Systems operating at 500, 360(2), 250, 220 and 150 MHz will be featured in the Facility and the following positions are now available:

SPECTROSCOPIST

We are seeking a highly motivated and talented individual who will assist in the design and construction of state-of-the-art Fourier transform NMR instrumentation. This work will initially involve development of a new 500MHz high-sensitivity spectrometer for NMR of solids and liquids. Previous experience in one or more of the following areas is essential: probe design for high-field FT NMR; spectrometer construction; mini/micro-computer programming and interfacing.

The primary job responsibility is to help establish and maintain a state-of-the-art NSF Regional NMR Instrumentation Facility for the Midwest in the School of Chemical Sciences. The successful candidate will typically possess a PhD in Chemistry or Physics, or an MS in Electrical Engineering. The starting salary will be not less than $22,000, depending upon experience and qualifications. The position is a continuing one and offers a month's vacation per year. The starting date is negotiable but an early date is preferred. The appointment will be academic/professional.

RESEARCH ASSOCIATE

We have an additional vacancy, for a Research Associate (about $12-13,000, with one month's vacation), to participate in the development of high-field NMR spectrometer and probe instrumentation, together with demonstration of improved performance on systems of chemical and biological interest. This position is available immediately, would be renewable for a second year, and would be suitable for a PhD chemist wishing to gain additional experience with NMR instrumentation and new applications.

Applications for either position should include a *curriculum vitae* and three letters of recommendation and should be submitted as soon as possible, preferably by January 1, to Professor Eric Oldfield, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801, Phone: (217/333-3374). The University of Illinois is an Affirmative Action/Equal Opportunity Employer.

W526(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF PHYSICAL SCIENCES

RESEARCH FELLOW IN ULTRAVIOLET PHYSICS

Applications are invited from suitably qualified persons for appointment as Research Fellow, to work with the Director, Professor J H Carver, in the Ultraviolet Physics Unit. The Unit investigates laboratory problems of astrophysical and atmospheric significance and collaborates with members of the Mount Stromlo and Siding Spring Observatories and the Plasma Research Laboratory of the Research School of Physical Sciences. Applicants should have research experience in one or more of the following fields: photoionization and photoabsorption in atmospheric gases, VUV spectroscopy, plasma spectroscopy, laboratory astrophysics, evolution of the atmosphere, space astronomy, computer control of atomic and molecular physics experiments.

Salary on appointment will be in accordance with qualifications and experience within the range: $A16,291 to $21,265 per annum. Present exchange rate: $A1 = £0.51p.

Appointment is for three years with possible extension to five years.

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on 31 January 1980. 3760(A)

MEDICAL RESEARCH COUNCIL

INSTITUTE OF HEARING RESEARCH

2 RESEARCH SCIENTISTS (Nottingham and Cardiff)

In collaboration with DHSS and the Welsh Office, the Institute is establishing outstations for multi-centre applied research into hearing, of relevance to services for the hearing-impaired. The multi-centre research and development work is of three main types, all using the close links with the service activity of the otolaryngology departments of the respective University Hospitals — epidemiology, detailed clinical characterisation, and the trial of pharmacological and electronic plus rehabilitative regimes of management. Experienced scientists are required to oversee the scientific aspects of the activity of the support teams provided by the Health Departments and to make an original personal research contribution within the broad programme of the Institute. The pre- and post-doctoral backgrounds of applicants may be in any science basic to medicine and its application, but some consideration will be given to the relationship between applicants' suggested personal research and the specific facilities in and outlined programmes for the respective outstations.

The appointments will be from 1st January 1980 or other time to be agreed. Further particulars and forms are available from Dr. M.P. Haggard, Institute of Hearing Research, Medical School, Clifton Boulevard, Nottingham NG7 2UH, to whom applications should be sent by 30th November 1979. Salary will be on the MRC Grade II or 1 scientist scale (£4232 to £6627 or £6886 to £8452). For an appointee of appropriate experience the senior grade (£8182 to £10,097) may be used. 3742(A)

NEW HALL
Cambridge

Applications are invited from school teachers who are teaching chemistry to spend a term at New Hall during the academic year 1980/81 as the Salters' Fellow Commoner. Free board and lodging and a small allowance is available.

Particulars may be obtained from the President, New Hall, Cambridge, to whom applications should be sent by 31st March, 1980. 3756(A)

ATHROFA GOGLEDD-DD CYMRU
The North East Wales Institute of Higher Education

SCHOOL OF NATURAL SCIENCE (RESEARCH DIVISION)
Kelsterton College

RESEARCH ASSISTANTS

Two posts are available, initially for a 2 year period, for a suitably qualified:
1. Biochemist, with interests in cellular and intermediary metabolism.
2. Cell biologist, preferably with experience in tissue culture techniques.

Both posts are industrially-sponsored, and form part of the Institute's programme of drug research, under the general supervision of Dr J C Allen, the Head of the Research Division. The Division possesses well equipped animal and tissue culture facilities.

Appointment will be on the T3/4 Grade of the NJC scales, with salary between £4,080 and £5,067 pa. Applicants, who should be Honours Graduates, and who will have the opportunity to register for a higher degree, should apply to The Institute Registrar, Kelsterton College, Connah's Quay, Clwyd, before 30th November, 1979. 3695(A)

UNIVERSITY OF WAIKATO
Hamilton, New Zealand

LECTURER IN EARTH SCIENCES

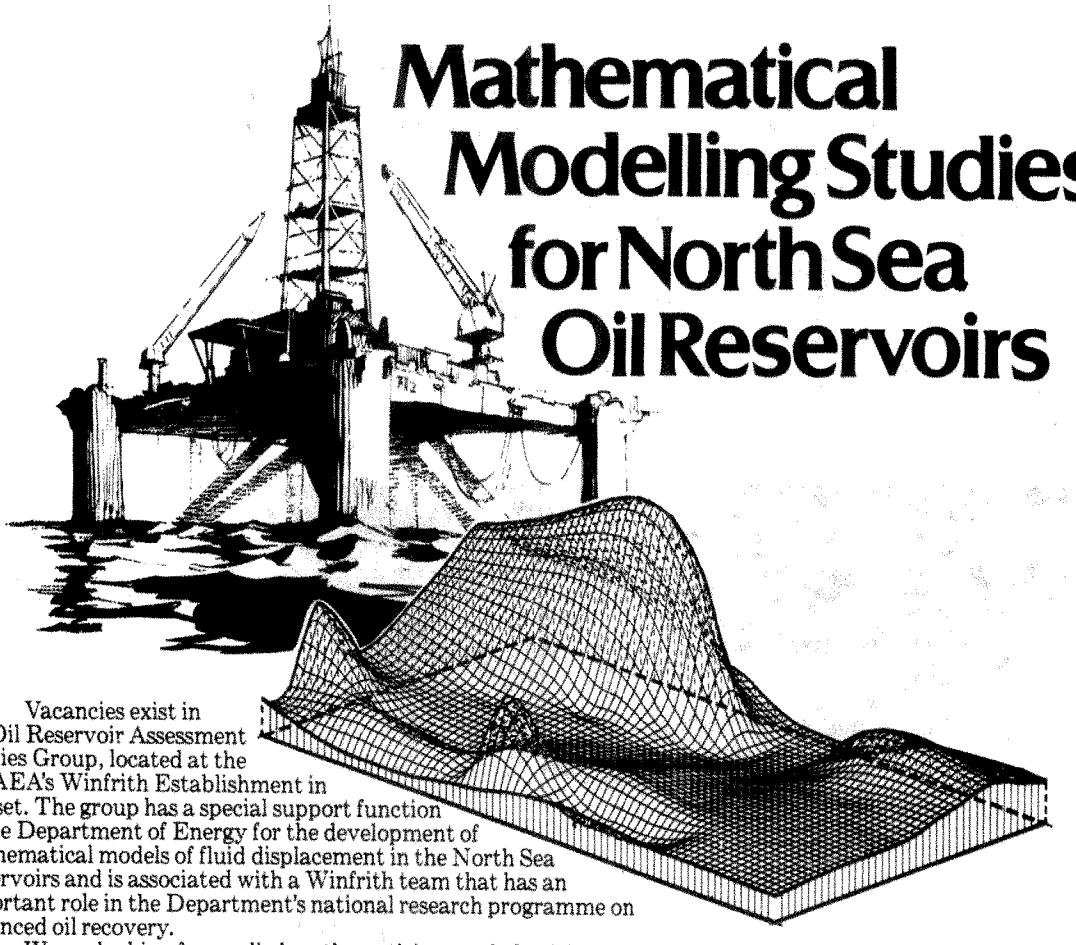
The University of Waikato invit applications for the position of Lecturer Earth Sciences. The appointment may made for a limited term. The appoint should be able to undertake and supervi research in either engineering hydrology in the physics and hydraulics of water an sediment motion in fluvial, estuarine, coastal environments.

Some general teaching in hydrology undergraduate level will also be require The salary will be in the range f Lecturers: NZ$13,722 to $16,861 p annum.

Further information on the method application and general conditions appointment is available from t Registrar, University of Waikato, Priv Bag, Hamilton, New Zealand or from Association of Commonwea Universities (Appts), 36 Gordon Squa London WC1H 0PF. Enquiries of academic nature may be made to Profes J D McCraw, Head of Earth Sciences the University. Applications close on January 1980. 3759(A

Applied Mathematicians and Physicists

Mathematical Modelling Studies for North Sea Oil Reservoirs



Vacancies exist in the Oil Reservoir Assessment Studies Group, located at the UKAEA's Winfrith Establishment in Dorset. The group has a special support function to the Department of Energy for the development of mathematical models of fluid displacement in the North Sea Reservoirs and is associated with a Winfrith team that has an important role in the Department's national research programme on enhanced oil recovery.

We are looking for applied mathematicians and physicists in their 20s or early 30s with honours degrees, preferably with some background in computational methods. Candidates should be able to demonstrate high-level scientific attainment.

The work spans a broad range of technical interests, including an understanding of fluid flow phenomena in porous media, solution of coupled systems of partial differential equations, computer code organisation and refinement, and an appreciation of field characteristics and the problems of optimal selection of model details in matching predicted performance to field observations. Simulation studies are undertaken on an IBM 3033 computer which is data linked via an Interdata 732 work station with colour graphics and other input/output devices.

Scientific Officer, Higher Scientific Officer and Senior Scientific Officer posts are offered with the recently agreed salaries effective from 1 January 1980 in the range of £4,720-£9,315, depending upon experience. The Winfrith establishment which undertakes a wide range of nuclear and non-nuclear programmes of work, is situated in pleasant rural surroundings close to the Dorset coast. There are excellent recreational and residential amenities in the surrounding areas from Bournemouth to Dorchester and Weymouth. We also have generous leave allowances and a contributory superannuation scheme.

For further information and application forms, please contact Mr. P. J. A. Fysh, Personnel Manager, AEE Winfrith, Dorchester, Dorset. Telephone No. 0305 3111, Ext. 3455. (Reference WA 321).

3738(A)

AEE WINFRITH

Head of Astrophysics Group

A vacancy has arisen for a Senior Principal Scientific Officer to head the Astrophysics Group of the new Space and Astrophysics Division within the merged Rutherford and Appleton Laboratories.

The current and planned programmes of the Division include ultra-violet and X-ray solar studies, ultra-violet astronomy and an infra-red astronomy satellite and millimetre astronomy project. The majority of the programme is based upon the use of Space techniques.

The Astrophysics Group, which is broadly identifiable with the former Astrophysics Research Division at Culham in Oxfordshire, undertakes programmes in collaboration with university research groups. In the future, these will involve a closer integration of the Laboratory's research and support roles.

The successful candidate will take overall responsibility for the work of the Group whilst pursuing his/her own research within the approved programme of the Group. He/she will report direct to the Division Head and be expected to play a full part in the management of the Division which currently has about 120 staff.

The officer appointed is likely to be an active research scientist with substantial experience in one or more of the areas outlined above and should have the ability to co-ordinate project teams and manage large collaborative programmes.

Salary will be paid within the range of £14,250 to £15,748 on the SPSO scale, according to experience. These rates will be payable from 1.1.1980.

The post will be based initially at Culham, but will move to the Rutherford Laboratory site at Chilton in Oxfordshire within one or two years.

Applicants should request an application form from:

Jane Griffiths, Rutherford and Appleton Laboratories, Chilton, DIDCOT, Oxon OX11 0QX.

Tel: Abingdon (0235) 21900 Ext. 510. Please quote reference VN5012.

CLOSING DATE: 3 December 1979.

3728(A)

Rutherford and Appleton Laboratories

COMMONWEALTH AGRICULTURAL BUREAUX

COMMONWEALTH MYCOLOGICAL INSTITUTE

Kew, Surrey

SCIENTIFIC INFORMATION OFFICER

The Commonwealth Mycological Institute has a vacancy for a Scientific Information Officer. The main duties include preparing abstracts of the literature on plant pathology and mycology, information retrieval from the CAB computerised database, preparation of bibliographies, and editorial work.

Qualifications: A degree in biological science in which plant pathology has featured is required. Post-graduate experience in plant pathology would be desirable, as would knowledge of or willingness to learn European languages.

Salary: In the scale £3,289 to £6,211 per annum plus Outer London Weighting £325, plus a compensatory allowance (taxable but not superannuable) of 3.3% of basic salary to offset personal contribution to superannuation scheme. Starting salary according to qualifications, experience and age.

Applications by 14 December to the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN, from whom application forms and particulars can be obtained. 3767(A)

LINCOLN COLLEGE (University College of Agriculture) New Zealand

LECTURER IN CROP PHYSIOLOGY

Applications are invited for the position of Lecturer in the Department of Plant Science. Applicants should have a postgraduate qualification and research experience in crop physiology, preferably combining a sound knowledge of physiological principles with personal experience of crop pasture production. Teaching at undergraduate and postgraduate levels will necessitate competence in such topics as photosynthesis, water relations, competition, etc.

Commencing salary according to qualifications and experience. Present salary scale for lecturing staff is: NZ$13,722 to NZ$16,861.

Travel and removal expenses are reimbursed up to specified limits. New Zealand Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University. Applications close on 15 January 1980. Please quote Vacancy No 78/50. 3720(A)

UNIVERSITY OF OXFORD

SOIL CHEMIST

preferably post-doctoral to work on an ARC project on the solubilization of soil phosphate in the rhizosphere. Salary in Grade 1A: £4333 to £7521.

Applications, mentioning two referees, to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF before 30 November 1979. 3750(A)

QUEEN ELIZABETH COLLEGE Kensington (University of London)

TECHNICIAN GRADE 5

NUTRITION DEPARTMENT

A vacancy exists for a technician to run the automatic analyser unit providing a service for the department and College. Applicants should have experience in the use of Technicon Autoanalyser systems, and some knowledge of amino acid analysis would be an advantage.

Salary in range £3,700 to £4,320 p.a. (under review) plus London weighting of £780 p.a. Application forms from the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH. Tel: 01-937 5411 ext 209. 3763(A)

Southampton THE UNIVERSITY

DEPARTMENT OF PHYSICS MAGNETISM GROUP

Two posts are available to support investigations into the magnetic properties of rare earth ions in (a) two-dimensional systems and (b) inter-metallic compounds. A third post (c) is available to study a number of transition metal alloys in high magnetic fields.

Applicants should hold or expect to hold a PhD in Physics or Materials Science, or for post (a) in Physical Chemistry although applications for posts (b) and (c) will be considered from those with a good honours degree. Such applicants would be permitted to register for a higher degree.

Starting salary will depend on age and experience and the posts will be available for two years in the first instance.

Applications which should include the names of two referees should be addressed to Professor E W Lee, Department of Physics, The University of Southampton, SO9 5NH, from whom further particulars can be obtained. Please quote reference N. 3740(A)

MANCHESTER POLYTECHNIC

DEPARTMENT OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT

to work in one of the following areas:- Toxicological and physiological effects of organic nitrates; copper tolerance in algae; biochemistry of peptide hormones; ultra-structure and physiology of mammalian capillary growth.

Candidates should be well-qualified academically and have research experience up to PhD level.

Salary scale: £3,780 to £4,083.

For application form (returnable by 7 December 1979) please send a self-addressed envelope marked "T/525" to the Secretary, Manchester Polytechnic, All Saints, Manchester, M15 6BH. 3724(A)

VIROLOGIST: SEARCH REOPENED Applications are solicited for a nine month, tenure track faculty position. Th appointment will be at the Assista Professor level and will be availab immediately. Applicants must have a Ph and postdoctoral experience. Th successful candidate will be expected develop and direct a quality researc program and advise graduate student Preference will be given to candidat actively studying viral diseases and vir pathogenesis but applications from indi iduals working in other areas will considered. Responsibilities will inclu teaching at the introductory and advanc levels.

Send curriculum vitae and three letters reference to: Neil R. Baker, Chairma Search Committee, Department Microbiology, The Ohio State Universi 484 W 12th Avenue, Columbus, Oh 43210. Applications will be accepted un March 1, 1980. An Equal Opportunit Affirmative Action Employer. qualified persons, including wome members of minority groups, and handicapped are encouraged to apply. W527(A)

NIHE

National Institute for Higher Education Dublin
Foras Náisiúnta um Ardoideachas Baile Átha Cliath

The National Institute for Higher Education, the most recently established higher education institution in Ireland, is engaged in recruiting its first academic, academic support and administrative staff to participate in the development and implementation of its first academic programmes which will commence in Autumn 1980 with the enrolment of its first students. The Institute will conduct programmes within the five Faculty areas of Administration, Business & Professional Studies, Science and Paramedical Studies, Engineering & Design, Education Studies and Communication and Human Studies.
Initially six first degree programmes will be conducted including programmes in Electronics, Analytical Science, Business Studies, Accounting & Finance, Computer Applications, Media Studies, Staff Development and Languages.

The Institute is now seeking to recruit to the following positions:-

Faculty of Science

Head of School of Chemical Sciences
Head of School of Biological Sciences
Head of School of Mathematical Sciences
Head of School of Physical Sciences

Two at Principal Lecturer level and two at Senior Lecturer level, together with 8 lecturers within the Schools.

CHEMISTRY
- Inorganic, Organic, Physical, Analytical.

BIOLOGY
- Botany, Zoology, Microbiology, Biochemistry, Environmental Science.

PHYSICS
- Applied Physics, Molecular Spectroscopy, Experimental Physics.

MATHEMATICS
- Applied Mathematics, Computer Science.

Faculty of Engineering

Head of School of Electronic Engineering *(Principal Lecturer)*
Four lectureships in Electronics (including Digital Electronics) are also to be filled.

Similar ranges of positions are also available within the Faculties of Administration, Business & Professional Studies, Communications & Human Studies and Education Studies.

Academic Support Services

A number of professional support units are also being established. The following positions are, therefore, also being filled.

Librarian *(Principal Lecturer)*
Head of Computer Services *(Senior Lecturer)*
Head of Educational Technology *(Senior Lecturer)*

CURRENT SALARY SCALES (under review)

Principal Lecturer	£10,859 -	£12,461
Senior Lecturer	£9,020 -	£11,309

Qualifications
Candidates will normally be expected to be highly qualified academically, preferably holding a higher degree or professional qualification, with considerable industrial, business and academic or research experience at an advanced level. Considerable importance will be attached to the teaching and research abilities of candidates.

For further information, apply immediately to: National Institute for Higher Education, 1 Lower Grand Canal Street, Dublin 2. Ireland. Telephone: 765175.

3733(A)

Biologist

Immunology Project Indonesia

As part of Australia's aid programme which seeks to assist developing nations in promoting the economic and social advancement of their people, the Australian Development Assistance Bureau (ADAB) is undertaking in Indonesia, a project for research and training in immunology. The Royal North Shore Hospital, Sydney will be managing agent for this project and is seeking the services of a Biologist.

The laboratories for the project are situated in the Biomedical Research Centre, National Institute for Health Research and Development (Ministry of Health), Jakarta. The research work of the project includes studies on the immunology of filariasis, hookworm and tuberculosis. The laboratories have a wide range of equipment for studies of cellular and humoral immunity.

Duties
The successful applicant will be responsible for much of the laboratory immunology, under the direction of the Australian team leader, Dr D A Higgins.

Qualifications and Experience
Applicants should have a PhD or equivalent qualification or experience in immunology or a relevant branch of biology.

Salary and conditions
The salary range is that for a Senior Scientific Officer (NSW Public Hospital Scientists Award), at present $A17,955-21,965. (At current rate of exchange £1 = $A1.88). Conditions of appointment include:—

* Appointment for two years in the first instance
* Return air fares to Jakarta
* Accommodation at a reasonable rental will be arranged
* Certain allowances, e.g. post allowance, cost-of-living adjustments, settling-in allowances, conference allowances
* Leave fares within the region

Applications
Full details of qualifications and experience including publications, should be sent to: Dr D S Nelson, Director, Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW, Australia 2065. A copy should also be sent to: Dr D A Higgins, Team Leader, Immunology Project — Indonesia, Australian Embassy, Jalan Thamrin 15, Jakarta, Indonesia. Enquiries about the work of the project and facilities available should be directed to Dr Higgins.

Applications close on 7 December, 1979. 3755(A)

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital) Watford Road, Harrow, Middx HA1 3UJ.

The division of Comparative Medicine requires a

VETERINARY GRADUATE

with an interest and experience in the health, welfare and management of experimental animals. The successful candidate will be expected to participate in collaborative projects and to carry out independent research to investigate animal models for the study of low birth-weight infants, particularly in relation to disturbances of carbohydrate metabolism.

This is a limited term post and the appointment will be for a period of 4 or 5 years depending on age and experience. Candidates should have a minimum of three years relevant experience since qualifying and salary will be within the range £6,066 to £8,992 plus £740 pa London allowance.

Potential applicants may discuss this appointment with Dr C R Coid (Tel: 01-864 5311 extn. 2510) before applying to Miss B. Shaw for an application form, quoting ref. 102/1/3896. Closing date 10th December, 1979. 3752(A)

AGRICULTURAL RESEARCH COUNCIL
INSTITUTE OF ANIMAL PHYSIOLOGY
Babraham, Cambridge, CB2 4AT

A vacancy, financed by the National Institutes of Health for three years from October 1st 1979, exists for a

POST DOCTORAL SCIENTIST

to assist with research on the mechanism of transport of steroids and other compounds into the seminiferous tubules of the testis.

Salary in Higher Scientific Officer (£4,727 to £6,211 p.a.) (£5,097 to £6,737 from 1.1.80) or Senior Scientific Officer (£5,876 to £7,864 p.a.) (£6,332 to £8,705 p.a. from 1.1.80) scale according to experience.

Candidates should have a first or upper second class honours degree in appropriate subject with at least 2 years for H.S.O. and 4 years for S.S.O. relevant post-qualifying experience and a PhD or equivalent training in cellular transport mechanisms, preferably in animals. Non-contributory superannuation schemes.

Application, quoting Ref. B.P.S.2. to the Secretary of the Institute by 30th November 1979. 3768(A)

UNIVERSITY OF NEWCASTLE UPON TYNE
CHAIR OF ORAL PHYSIOLOGY

Applications are invited for the Chair of Oral Physiology which will become vacant on the retirement of Professor G N Jenkins on the 30th September, 1980. The post is open to candidates, with or without dental qualifications, who have biochemical or physiological research interests. The appointment to the Chair will carry with it the Headship of the Department of Oral Physiology for an initial period of five years.

The salary will be in accordance with the professorial scale with membership of the appropriate superannuation scheme.

Further particulars may be obtained from the Senior Assistant Registrar, The Medical School, University of Newcastle upon Tyne, Queen Victoria Road, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies) giving the names of not more than three referees, must be lodged not later than 15th January, 1980.

(Applicants from outside the British Isles may submit one copy only.) 3726(A)

SUNDERLAND POLYTECHNIC
Faculty of Science
SCHOOL OF PHARMACY
RESEARCH OFFICER/ RESEARCH ASSISTANT IN PHARMACOLOGY

Applications are invited from interested persons for the above position, renewable annually for up to three years. The successful applicant will be expected to work on a programme, funded jointly by Fisons Limited, Pharmaceuticals Division and the Polytechnic, to study the pharmacology of chemical mediators of allergic disease in abdominal organs. There could be an opportunity for suitably qualified persons to register for a higher degree (MPhil/PhD, CNAA).

Research Officer: £3,480 to £3,780
Research Assistant: £3,030 to £3,330.

An application form and further particulars may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland, SR1 3SD, and should be returned as soon as possible. 3722(A)

THE UNIVERSITY OF PAPUA NEW GUINEA

Applicatins are invited for the post of

LECTURER

in the Department of Biology. The appointee will be required to teach courses in Plant Physiology and Ecology and contribute to the general teaching of Botany especially with regard to the lower plants. Tropical experience is an asset but not essential. There is particular opportunity for applicants interested in the biology of aquatic plants.

Salary scale; Lecturer II K13725 pa Lecturer I K11875 pa (£1 sterling = K1.5) Family passages; baggage allowance; 3 yr contract; 24% gratuity of which 5% taxable; research support; rent free accommodation; overseas leave; educational allowances; medical scheme; secondment welcomed.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Assistant Secretary, P.O. 4820, University PO, Papua New Guinea by 31 December 1979.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT.

Further details may be obtained from either address. 3771(A)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF BIOCHEMISTRY

RESEARCH OPPORTUNITIES

Applications are invited for the following positions which are available under the direction of Drs. J. Kay and A. Cryer.

RESEARCH ASSISTANT Grade 1B

to work with Dr Kay on chicken pepsin as a rennet substitute. Studies on its production, stability and activity. The project will involve techniques such as enzyme purification, affinity chromatography, milk clotting assays and protein structural investigations.

The appointment which will be for one year in the first instance, with the possibility of renewal for a further two years, is financed by a grant from the Agricultural Research Council. The salary will be in the range of £3,775 to £4,333 (under review).

Candidates should have a good Honours degree in biochemistry or a related subject and research experience in protein chemistry/enzymology would be an advantage.

POST-DOCTORAL RESEARCH ASSISTANT Grade 1A

o work with Drs Kay and Cryer in collaboration with Professor R F Mahler of the Clinical Research Centre on cellular studies of spontaneous atherosclerosis in White Carneau pigeons. The project will nvolve the characterization of both protein and glycosaminoglycan synthesis in pecific cell lines maintained in culture.

The appointment which is for two years with possible extension for a third, is inanced by a grant from the British Heart oundation. The salary will be £4,333 for he first year and £4,622 for the second. xperience of cell culture techniques and adioisotope methodology as applied to rotein and polysaccharide biosynthesis easurements would be an advantage.

POST-DOCTORAL RESEARCH ASSISTANT Grade 1A

o work with Dr Cryer on molecular and ellular aspects of the control of cardiac poprotein lipase activity. The project will volve studies of enzyme synthesis in olated cardiac muscle cells maintained in n already established incubation system.

The appointment which is for one year in e first instance with the possibility of enewal for a further two years, is financed y a grant from the Medical Research ouncil. The salary will be in the range 4,333 to £4,910 (under review).

Candidates should have a PhD or quivalent post-graduate research perience. Experience of work with olated cells, immunological techniques or neral metabolic studies would be an vantage.

Applications (2 copies), including a rriculum vitae together with the names d addresses of two referees and dicating clearly the post of interest, ould be sent to the Vice-Principal dministration) and Registrar, University llege, PO Box 78, Cardiff CF1 1XL. osing date 13th December, 1979. ference 1925. 3731(A)

Analytical Biochemist or Pharmacologist for quality control

The Radiochemical Centre Limited is a world leader in the production and marketing of radioactive isotopes for medical, scientific and industrial purposes. Although the main laboratories and head office of the Company are at Amersham, Buckinghamshire, a major new establishment is nearing completion in Cardiff.

We now require a versatile Life Scientist to be based at Cardiff and who will take responsibility for the development of new quality control testing procedures, including biological methods, for a wide variety of radioactive biochemicals including amino-acids, nucleotides, steroids and biochemicals used in receptor site studies. This is a new post offering an opportunity to employ some of the advanced techniques used in current academic biochemical research.

The position will appeal to Life Scientists – men or women who have had experience and/or research training in analytical biochemistry, pharmacology or biochemical research.

Conditions of service are excellent and include good holidays and a contributory pension scheme. Salaries which are **at present under review** currently start at around £5,000 - £6,000 pa dependent on the calibre of the successful applicant. They are increased with service in accordance with incremental scales. An initial period of training at our Amersham headquarters will be required.

Please write quoting reference number CR73 giving concise career details to: Mr. R. Hart-Jones, Personnel Department, The Radiochemical Centre Limited, Forest Farm, Whitchurch, Cardiff.

The Radiochemical Centre
Cardiff

3739(A) 1979

nature

London
3 Dyers Buildings
London EC1N 2NR
01-831 6901

New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

BIOLOGY TEACHING OVERSEAS

Research-Teaching positions in Biology starting Fall 1980 in such specialties as: Plant Physiology, Developmental Biology, Plant Taxonomy, Marine Biology (Invertebrate Zoology/Ecology) PhD required, rank and salary dependent on experience & qualifications. Normal contract 3 years with fringe benefits.

Air mail CV, resume & letters of recommendation to: Chairman, Biology Dept. American University of Beirut, Beirut, Lebanon. Review begins Feb. 1, 1980.

AUB is an equal opportunity affirmative action employer. W534(A)

The Department of Pathology at the University of Pittsburgh School of Medicine wishes to recruit a member for its Division of Experimental Pathology. The position will entail research and teaching to medical students and to graduate students. There are ample clinical opportunities for research programs needing them. The major areas of interest in the Division are immunology and tumor biology.

The level of academic appointment will be commensurate with the candidate's qualifications.

Reply with Curriculum vitae to: Thomas J. Gill, MD, University of Pittsburgh School of Medicine, Department of Pathology, Room 716, Scaife Hall, Pittsburgh, PA 15261.

An equal opportunity/affirmative action employer. W495(A)

UNIVERSITY OF EDINBURGH

Department of Molecular Biology

POSTDOCTORAL RESEARCH FELLOW

required as soon as possible to work on the molecular relationships of plasmids from strains of *Pseudomonas* and of enterobacteria. The methods currently being used include genetic manipulation *in vitro*, DNA-DNA hybridisation and electron microscopy of heteroduplexes. The position is for two years.

Further information from Dr P M A Broda (tel 031-667 1081, ext 2886) to whom applications should be sent with the names of referees and details of experience. Please quote reference 5063. 3725(A)

ELECTROPHYSIOLOGIST

Syntex Research Centre is the research division of Syntex Pharmaceuticals Limited, part of the multi-national Syntex Corporation.

Due to continued expansion we have a vacancy for an electrophysiologist within the Pharmacology Department. This position will involve the setting up of an electrophysiology laboratory to investigate the effects of cardiovascular and CNS active compounds. Ideally the suitable applicant will be recently qualified PhD with a keen interest in mode of action studies. Applicants about to attain PhD would be considered.

We offer salaries competitive with appropriate public service grades and attractive conditions of service which include non-contributory pension scheme, free life insurance, membership of BUPA, bonus opportunities and assistance with relocation expenses.

The Centre is in pleasant surroundings in the Research Park of Heriot-Watt University, 7 miles west of the centre of Edinburgh.

Please write or telephone for an application form to: Mr A B Cran, Director of Personnel at the address below.

SYNTEX Research Centre

Research Park
Heriot-Watt University
Riccarton
Edinburgh
EH14 4AS

031 449 6211

3753(A)

Range Animal Nutritionist

Overseas Assignment

The Kuwait Institute for Scientific Research is a rapidly expanding institute for applied science and technology. We are currently seeking a Researcher who is interested in a challenging career in range animal nutrition.

You should have a Ph.D. and previous experience in range animal nutrition, preferably dealing with sheep. We also require a minimum of 2-4 years' experience in range animal studies and academic training in an arid region plus a thorough knowledge of laboratory and field techniques of range/sheep studies.

Kuwait Institute offers attractive salaries commensurate with qualifications and experience, furnished housing, liberal fringe benefits including round trip air tickets and free medical benefits.

Please airmail your complete resume by **January 1, 1980** to: **Mr. Habib Al-Sahhaf, Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, Kuwait, State of Kuwait.**

Kuwait Institute for Scientific Research

W523(A)

TECHNICIAN/ RESEARCH OFFICER

required at the Imperial Cancer Research Fund Human Tumour Immunology Group, University College Hospital Medical School, London, WC1 to work on production and use of monoclonal antibodies to human tumour and differentiation antigens. Experience in tissue culture or cellular immunology an advantage, HNC/Degree.

Salary range £4714 to £5949. For further information and application form write or telephone Miss S.M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2 on 242 0200 ext 305. 3741(A)

DALHOUSIE UNIVERSITY
Halifax, Nova Scotia, Canada

PHYSICAL OCEANOGRAPHERS

Grant-supported positions available for two research associates for theoretical and experimental work on shelf processes. Both post-doctoral and senior applicants welcome. Submit applications with names of three referees before December 15.

Further details available. Dr. C. Garrett, Department of Oceanography, Dalhousie University, Halifax, N.S., Canada, B3H 4J1. W530(A)

FACULTY POSITIONS
DEPARTMENT OF GEOLOGY
UNIVERSITY OF WINDSOR

Applications are invited for two tenure-track positions at the Assistant Professor rank in the following fields: 1. Paleontology-Biostratigraphy, and 2. Engineering Geology — Geotechnical Engineering.

Applicants with expertise in closely related applied geological disciplines (e.g., paleoecology, petroleum geology, rock mechanics, clay mineralogy and oxygen isotope geology) are encouraged to apply. They should hold a doctoral degree or anticipate receiving it near the time of commencement of the positions on July 1, 1980. Both positions involve teaching at the undergraduate and graduate levels as well as conducting a research programme involving undergraduate and graduate thesis students. Salary will depend on the applicant's experience in teaching, research and industrial experience and will be in the range of $20,000 to $25,000. Send a curriculum vitae and the names and addresses of three referees to: Dr D T A Symons, Department of Geology, University of Windsor, Windsor, Ontario, Canada N9B 3P4. W533(A)

UNIVERSITY COLLEGE LONDON
DEPARTMENT OF PHYSIOLOGY

Applications invited for position of

POSTDOCTORAL RESEARCH ASSISTANT

in a Developmental Neurobiology Programme supported by the Wellcome Trust. Research is in progress to study the internal environment of the fetal brain in relation to brain development. Two year appointment to work in group headed by Dr N R Saunders. Starting date 1 December 1979 or by arrangement. Experience of morphological techniques essential, preferably including immunohisto-chemistry.

Salary range £4,910 to £5,199 plus £740 London Allowance.

Further details from and applications to: Dr N R Saunders, Dept. of Physiology, University College, London, Gower Stret, WC1E 6BT. 3770(A)

SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING
SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

A Scientific Officer is required to join the Cereals Department at the Scottish Plant Breeding Station.

The appointee will be required to join a group working with spring barley and spring oats with the object of producing crop varieties adapted to local conditions. Training in genetics and/or pathology along with an interest in the practical aspects of plant breeding would be an advantage.

Qualifications: A pass degree or HNC with relevant experience or an honours degree in a biological science. At least five years relevant post-graduate experience is normally required for appointment as HSO.

Salary: Depending on qualifications and experience:
Scientific Officer: In the range £3,289 to £5,085 per annum.
Higher Scientific Officer: In the range £4,726 to £6,211 per annum.
The post is superannuable.

Application forms and further information may be obtained from the Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF, to whom completed application forms should be returned not later than 3rd January 1980. 3735(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
DEPARTMENT OF PHYSICS

IMAGE ANALYSIS AND IMAGE PROCESSING

Applications are invited for the following posts which are tenable for periods of up to three years on funds provided by the Science Research Council. The appointments will date from 1 January, 1980, or as near as possible thereto and will be made at a salary commensurate with age and experience.

POST-DOCTORAL RESEARCH ASSISTANT

The work concerns the digital analysis and enhancement of images obtained by electron and optical microscopy or from earth satellite recordings. Appropriate experience in computing is required, in the context of image processing, to play a leading role in soft-ware development within a scientific programme to achieve the benefits of array processing — for example, in the simultaneous processing of multiple images. Digital processing facilities are available using a local dedicated GEC 4070 computer system with interactive graphics and remote access to an ICL Distributed Array Processor.

Salary in range £4,333 to £6,066 pa plus London allowance of £740 p.a.

COMPUTER PROGRAMMER/ COMPUTER SCIENTIST

A graduate with experience of programming is required to run a GEC 4070 computer system with interactive graphics for the image analysis group and to provide programming support for the work described above. Part-time registration for a higher degree may be arranged for a suitable candidate.

Salary in range £3,775 to £4,333 p.a. plus London allowance of £740 p.a.

Applications, giving two referees, to Professor R.E. Burge, Physics Department, Queen Elizabeth College, Campden Hill Road, London, W8 7AH.

3762(A)

Wellcome

Molecular Biologists

Scientists and Technicians

with relevant experience are invited to apply for positions at several levels within a young enthusiastic cell biology group with the objective of utilizing the potential of recombinant DNA techniques to contribute to problems of medical and veterinary interest.

Candidates should have a relaxed attitude to collaboration together with the motivation and ability to make a significant contribution to a major basic research programme studying the genetic properties of viruses, bacteria and parasites with a view to engineering microbial hybrids capable of expressing biological molecules of potential importance in immunoprophlaxis.

Excellent facilities are available, including containment laboratories from category II to category IV.

Salaries will be in the range £6,000 to £11,000 for scientists and £4,000 to £7,000 for technicians, depending on age and experience.

The Laboratories are situated in pleasant parkland surroundings close to open countryside yet within easy reach of Central London. Conditions of employment are excellent and include subsidised canteen, pension scheme, 4 weeks' holiday, relocation expenses where appropriate and wide-ranging sports and social facilities.

Please apply with full Curriculum Vitae including publication list, to:

Personnel Manager, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

3748(A)

FACULTY POSITION IN THE DEPARTMENT OF ASTRONOMY, HARVARD UNIVERSITY

The Department of Astronomy at Harvard University plans to make one or two appointments to the position of Assistant Professor to begin in the Summer or Fall of 1980. The department invites applications for these positions, which will have a term of three to five years. The Department wishes to consider applications for highly qualified persons in any area of astronomy or astrophysics, and it particularly encourages applicants in the areas of theoretical astrophysics, high energy astrophysics, and optical astronomy. The successful applicants will be expected to participate actively in teaching activities within the Department of Astronomy, and it is also expected that they will spend a significant fraction of time in research activities at the Center for Astrophysics.

Letters of application should be accompanied by a résumé of experience in teaching and research, a statement of research interests and a bibliography. The applicant should request three astronomers or physicists to write directly to Professor A.G.W. Cameron, Chairman, giving their evaluation of the applicant's abilities. Applicants are expected to have received their PhD at least one year before taking up this post.

Harvard University is an Equal Opportunity Employer with an Affirmative Action Program, and it encourages applications from women and members of minority groups.

Send applications to the Department of Astronomy, Harvard University, 60 Garden Street, Cambridge, Massachusetts 02138 for receipt by February 1, 1980.

W532(A)

ODENSE UNIVERSITY
Denmark
PROFESSOR IN PHOTOSYNTHESIS

We again invite applications for the new position as professor in Photosynthesis at the Institute of Biochemistry. The position is available for occupation as soon as possible.

It is desired to appoint a person, whose research activities are experimental investigations of the functions of the photosynthetic membrane systems in eukaryotes and/or prokaryotes. A part of the current research in the Institute is concerned with electron transport in respiratory and photosynthetic systems, and the Institute is well equipped with special apparatus for this type of work.

The successful applicant will be expected to participate in the teaching programme of the Institute, which includes elementary courses common to science and medical students, advanced courses for science students and supervision of advanced project work.

Further information is obtainable from Institute of Biochemistry, Odense University, telephone (09) 15 86 00, ext. 2441.

The application should contain information about the candidate's teaching experience.

The employment field covers the Ministry of Education and the institutions under it. The wage frame is 37, and the salary amonts to Danish Kroner 230.520,24 a yer inclusive of bunusses as per the 1st October, 1978.

A professional selection committee will discuss the applications and the recommendations will be sent out to all applicants in its complete form.

Applications received until now will be taken into consideration if they are not withdrawn.

Application in 5 copies, marked "Position No. 745" enclosing curriculum vitae and documentation for professional and pedagogical activities must be made to the Queen and sent together with all enclosures also in 5 copies to: Journalkontoret, Odense University, Campusvej 55, DK 5230 Odense M, Denmark, so that we will receive it on the 2nd January, 1980 as the latest.

W522(A)

Research Scientist

Guinness Research Laboratory

If you have already gained your doctorate in Chemistry or Biochemistry, backed by two years' experience in protein or polyphenol chemistry, then Guinness have a career opportunity for you at their Park Royal Brewery.

Researching in the field of polyphenol, carbohydrates and lipid interaction, you will be responsible for planning the work involved in both fundamental research and in the development of projects to strict time schedules.

Your work will involve you in the Guinness brewing group activities in the UK, as well as the possibility of overseas travel on specific assignments for the Guinness brewing operation overseas.

Not only should you be an initiator, capable of highly creative thought, but you should have the ability to motivate others and exploit your basic and applied research.

Our conditions of employment are excellent, with a salary from £7,350, 5 weeks holiday, profit share, non-contributory pension scheme and first-class opportunities for career development within this diverse and expanding group.

Please write or telephone for an application form to:
Miss A. P. Lloyd, Assistant Personnel Manager, Arthur Guinness Son & Co. (Park Royal) Ltd., Park Royal Brewery, London NW10 7RR.
Tel: 01-965 7700.

GUINNESS

3766(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF GENETICS

Applications are invited for a post of

RESEARCH FELLOW

in the above Department. The successful candidate will work on a project, supported by the SRC, on the biosynthesis and mode of action of cytokinins in the moss, *Physcomitrella patens*. Applicants with research experience in either plant physiology or microbial genetics would be especially suitable. Candidates should possess a PhD degree. The appointment will be made for a fixed period of up to 22 months from 1 January 1980 or from a date to be arranged.

Salary at an appropriate point on the 1A scale for Research and Analogous Staff (£4333 to £7521) (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 50/5/D. Closing date for applications 1 December 1979.

3744(A)

GUY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS

Pre- or post-doctoral Research Assistant (Biochemist or Immunologist), with experience in biochemistry or immunochemistry of bacteria, required for one year to work on research project concerned with separation of specific components, especially proteins from Streptococci. Salary according to qualifications, with superannuation.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref IM7. 3729(A)

SECTION LEADER
Cardiovascular Research

A vacancy exists within the pharmacology department for an experienced scientist to lead a team of seven graduates and technicians engaged in research into drugs for use in hypertension and other cardiovascular disorders.

The ideal candidate will hold a PhD and have gained a minimum of 2 years post doctoral experience in cardiovascular physiology or pharmacology in either industry or an academic institution. After an initial induction period he/she will be expected to make a positive contribution to the work of the department and will be actively encouraged to publish scientific results.

The present salary range, £6,500 to £7,500, will be reviewed in January. Other benefits are commensurate with the level of appointment.

The company's modern research laboratories are situated in a pleasant part of the Thames Valley and have the added advantage of being within easy access of the M4.

For further details and an application form please contact:

Miss C. Bradshaw, Personnel Officer,
WYETH LABORATORIES, Huntercombe Lane, South, Taplow, Maidenhead, Berks SL6 0PH.

Wyeth Laboratories 3749(A)

DIRECTOR OF BIOLOGICAL RESEARCH (CANADA)

The Canadian affiliate of Merck & Co. Inc.'s world-wide research organization has a challenging senior research position available as Director of Biological Research in Canada. This position reports to the Canadian Executive Director of Research, and is responsible for the effort of a team of approximately 25 scientists involved in CNS and respiratory research.

The ideal candidate we are seeking will have a PhD degree in the biological sciences, 10-15 years industrial or university research experience and a proven record of scientific achievement and recognition. Additionally, the candidate will possess strong leadership and communication skills and a demonstrated ability for creative thinking.

This senior research position offers an exceptional opportunity for growth and development within one of the most progressive industrial research organizations in the health care field in Canada. Excellent salary and full range of employee benefits.

Our research laboratories are located in modern facilities in Kirkland, Quebec, a suburb 15 miles from downtown Montreal.

Please send a curriculum vitae, a list of publications and the names of three references to: The Employment Manager, Merck Frosst Laboratories, PO Box 1005, Pointe Claire-Dorval, Quebec, Canada, H9R 4P8. W531(A)

MFL

Laboratoires **Merck Frosst** Laboratories

IMPERIAL COLLEGE
(University of London)
DEPARTMENT OF BIOCHEMISTRY
POSTGRADUATE ASSISTANT IN NEUROCHEMISTRY

Applications are invited for the above position to work on the putative role of neuropeptides in pain processes, under the direction of Professor J Hughes and Dr H Morris. The work will involve the purification and determination of peptides in cerebrospinal fluid obtained in clinical studies and candidates should have a biochemical/ physiological training. The post, which is funded by the Wellcome Foundation is available for a maximum of three years, at a starting salary on research grade 1B of £4,333, plus London allowance of £740 pa.

Applications including curriculum vitae and names and addresses of two referees should be sent to Professor J Hughes, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ. 3761(A)

KINGSTON POLYTECHNIC
SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES
RESEARCH ASSISTANT IN HETEROCYCLIC CHEMISTRY

The work, supervised by Dr D T Hurst, will be concerned with the synthesis of pyrimidines and related compounds, the electrophilic substitution of pyrimidines, and studies of physico-chemical and biological properties of such compounds.

Applicants should have an honours degree, GradRIC or equivalent qualification, and if successful, will be expected to register for a higher degree.

Salary range £3,174 to £3,462 inclusive. Further details and application forms from Dept AO, Academic Registry, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. 3696(A)

Wellcome

The Wellcome Research Laboratories
Beckenham
Department of Immunochemistry

Senior Technician
£4,084 — £5,476

A Senior Technician is required for work in genetic engineering. Candidates, who should possess an HNC or equivalent, should have experience in growing, harvesting and the sterile handling of micro-organisms. Some experience in biochemical techniques including cell fractionation, purification of macromolecules and gel electrophoresis would also be an advantage.

The Wellcome Research Laboratories are situated in pleasant parkland surroundings within easy reach of Central London. Excellent conditions of employment include subsidised canteen, pension scheme, four weeks' holiday and sports and social facilities. Generous relocation expenses are available where applicable.

For an application form please write to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, giving brief details of your background and experience.

3747(A)

Please mention
Nature
when replying to these advertisements

Public Health Laboratory Service
Central Public Health Laboratory

Biochemist

Applications are invited from suitably qualified persons, AIMLS or BSc, to join a research group studying the etiology of large bowel cancer. Work will include routine use of thin layer chromatography and gas-liquid chromatography.

NHS terms & conditions of service. Salary scale (from 1.1.80) in range £3807 to £6075 + London Weighting.

Microbiologist

A basic grade microbiologist is required in the Food Hygiene Laboratory. Applicants should have an honours degree in Microbiology and some post-graduate experience would be an advantage. The successful applicant will be concerned with the general work of the laboratory, the isolation and identification of **Clostridium botulinum** and investigations on gastro-enteritis of unknown aetiology.

Salary according to age, qualifications and experience, on the NHS Whitley Council Scientific Scale plus London Weighting.

Applications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT. Tel: 01-205 7041.

PHLS

3754(A)

Recombinant DNA Research

GENENTECH, INC. is continuing to expand its research program and is inviting applications for several positions in recombinant DNA research. Positions are available for highly qualified individuals in the areas of molecular genetics, molecular and general virology, immunology, and Bacillus and yeast molecular biology at the following levels:

SENIOR POSITIONS: Highly motivated individuals with a PhD and extensive postdoctoral experience are sought for project-and/or group-leader positions. Candidates should have the drive and capability to set up and direct independent research programs.

POSTDOCTORAL POSITIONS: Under its postdoctoral program, Genentech offers two-year appointments at competitive salaries in a stimulating scientific environment. We strongly encourage our scientific staff to publish.

RESEARCH ASSOCIATES & TECHNICIANS: Candidates for these positions should have a BS or MS degree, significant laboratory experience and the ability to carry out a successful research program with minimal supervision.

Genentech is situated on the San Francisco peninsula and provides a stimulating working environment with competitive salaries and extensive fringe benefits. Please send a resume and list of three references to the attention of Mrs. Chris McKinley at:

GENENTECH, INC.
460 Pt. San Bruno Blvd.
So. San Francisco, CA 94080

W529(A)

BIOCHEMIST

We have a vacancy for a biochemist with experience in enzymology or protein biochemistry to investigate the interaction of new chemical entities with various biochemical systems. The successful man/woman will work in close liaison with chemists and biochemists, and is expected to have considerable input into the overall design and direction of the work.

The position is suitable for a biochemist with 2 or 3 years post-doctoral experience. The post is in the Department of Biochemical Pharmacology at Fisons Research and Development Laboratories in Loughborough, Leicestershire.

We offer a competitive salary depending upon qualifications and experience. Benefits include 4 weeks holiday, an excellent contributory pension scheme which includes life assurance, a discretionary annual bonus, and generous assistance with relocation expenses where appropriate.

For an application form please write or ring, quoting reference number RD73/N: Miss C L Mason, Personnel Officer, FISONS LIMITED, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire LE11 0QY. Tel: Loughborough 66361

3743(A)

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TUTORS IN CHEMISTRY

Applications are invited for appointments as Tutor in Chemistry in the School of Chemistry, tenable not before 1 February, 1980. Appointments will terminate on 31 January, 1981, with a possibility of further annual renewal.

Candidates should possess a higher degree in any of the major fields of Inorganic, Organic, Physical or Theoretical Chemistry. One appointment will be in the field of Physical Chemistry. In addition to teaching duties, the successful applicant will be expected to play an active part in research in the School.

Salary will be within the range $A11,579 to $A13,633 per annum.

Applications, including curriculum vitae, together with the names and addresses of three referees should be lodged with the Registrar, Macquarie University, North Ryde, 2113, NSW, Australia. Applications close on 30 November, 1979. 3746(A)

FACULTY POSITION IN BIOLOGICAL SCIENCES
at the Hopkins Marine Station of Stanford University

Applications are invited for an appointment in the Department of Biological Sciences of Stanford University, to be resident at the Hopkins Marine Station in Pacific Grove, California. The position is without restriction as to rank although some preference will be given to younger candidates.

The position involves both teaching and research. The areas in which the Station wishes to expand its faculty include Cellular, Neuro- and Developmental Biology. All applications must include a curriculum vitae, a statement of research interests and a list of three people from whom letters of reference will be sought.

Applications should reach C S Pittendrigh, Hopkins Marine Station, Pacific Grove, CA 93950 by January 15, 1980. Stanford University is an equal opportunity employer. W505(A)

ROTHAMSTED EXPERIMENTAL STATION
Harpenden, Herts AL5 2JQ
PLANT PATHOLOGIST

required for a 1-year post financed by the Potato Marketing Board to work on the bacterial diseases of growing and stored potatoes.

Minimum qualifications: HNC, pass degree or equivalent qualification in Plant Pathology, Botany or Microbiology.

Appointment in grade of Scientific Officer, £3,289 to £5,085. (Scale rises 1.1.80 to £3,591 to £5,486 pa). Placing on scale depends on qualification and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 413 by 30th November, 1979. 3736(A)

FACULTY POSITION
Organic Chemistry

Applications are invited for a tenured senior appointment which the Department of Chemistry at Stanford University expects to be able to make in the near future. Applicants must have outstanding research credentials in organic chemistry and a strong commitment to teaching undergraduates as well as graduates.

In applying, send curriculum vitae and bibliography to: Professor K.O. Hodgson, Department of Chemistry, Stanford University, Stanford, California 94305. Stanford is committed to Equal Opportunity through Affirmative Action in employment and we are especially eager to identify women and minority persons with appropriate qualifications.

Applications should be received by 15 December, 1979. W516(A)

GRANTS and SCHOLARSHIPS

THE ROYAL SOCIETY
BROWNE RESEARCH FUND
MAURICE HILL RESEARCH FUND
MARSHALL AND ORR BEQUEST
Grants 1980

The Council of the Royal Society invites applications from qualified research workers for personal grants:

(a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

(b) from the Maurice Hill Research Fund (established in memory of the late Dr M N Hill, FRS, for the encouragement of research in physical oceanography and marine geophysics) for research at sea and/or in the laboratory in physical and chemical oceanography, marine geophysics and geology.

(c) from the Marshall and Orr Bequest for the support of marine biological research.

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them. Recipients are permitted to use grants while receiving salaries from regular appointments.

In 1980 the total amounts available to be shared among all successful applicants will be about £4,500 from the Browne Research Fund, £9,500 from the Maurice Hill Research Fund and £7,500 from the Marshall and Orr Bequest; individual grants will not ordinarily be more than £750.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by 15 December 1979. 3607(H)

COURSES

NATO ADVANCED STUDY INSTITUTE

"MOLECULAR ELECTRO-OPTICS. ELECTROOPTIC PROPERTIES OF MACROMOLECULES AND COLLOIDS IN SOLUTION — THEORETICAL STUDIES, EXPERIMENTAL METHODS, AND EXPERIMENTAL RESULTS"

July 14-24, 1980
at Rensselaer Polytechnic Institute, Troy, N.Y., USA

Closing date for applications: March 15, 1980

This NATO Advanced Study Institute will cover the theory of molecular electro-optics and rotational diffusion, the interaction of chemical and biological systems with electric fields, the instrumentation used in molecular electro-optics, applications of electro-optics to proteins, polypeptides, polynucleotides, nucleoproteins, polyelectrolytes, colloids and liquid crystals, and an introduction to electrophoretic light scattering and to magneto-optics. Lectures include O'Konski (Univ. of Calif, Berkeley), Jennings (Brunel Univ.), Buckingham (Cambridge Univ.), Neumann (Max-Planck Inst., Munich), Garcia de la Torre (Univ. Extremadura), Stoylov (Bulgarian Acad. Sci.), Charney (Natl. Inst. of Health, Bethesda), Weill (Strasbourg), Jernigan (Natl. Inst. of Health, Bethesda), Houssier (Univ. Liège), Greve (Vrije Univ., Amsterdam), Pörschke (Max-Planck Inst., Am Fassberg), and Uzgiris (General Electric).

A limited number of grants are available to participate from NATO countries. Except in extraordinary circumstances, they will cover only about 50% of living and travel expenses. Living expenses are $310.

Applications may be in the form of a letter including curriculum vitae and present scientific interest to: Professor Sonja Krause, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181, USA. W528(D)

ASSISTANTSHIPS

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BIOCHEMISTRY
POSTGRADUATE RESEARCH ASSISTANTSHIP

Applications are invited for a Wellcome Trust funded post to investigate the synthesis and function of a multivalent receptor in *Escherichia coli*.

The grant is for a period of three years and the starting salary will be £3,775 on the Range 1B scale. The successful applicant will be expected to register for a PhD.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr Robert E Glass, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH. 3737(P)

FELLOWSHIPS and STUDENTSHIPS

Southampton THE UNIVERSITY

SOLAR CELL THEORY

Post-Doctoral SRC Fellowship

Applications are invited for the above post-doctoral post which is available from January 1980, and is suitable for appropriately interested mathematicians, physicists or engineers. Salary up to £4505 per annum.

Applications should be sent to Professor P T Landsberg, Department of Mathematics, University of Southampton, Highfield, Southampton SO9 5NH, before 20 December. Candidates are asked to request two referees to write directly to the same address. Please quote Ref: N. 3764(E)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES
POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral fellowship to work under the supervision of Professor W D P Stewart on *The Physiology and Biochemistry of Associative Symbioses between Eukaryotic Plants and N_2-Fixing Blue-Green Algae (Cyanobacteria)*. The post is for a maximum of two years.

The salary will be within the range £4333 to £4910 (under review). Superannuation under USS.

Applications should be sent to The Secretary, The University, Dundee DD1 4HN by 6/12/79. Please quote reference EST/95/79H. Informal enquiries may be made to Professor W D P Stewart, FRS, at the Department of Biological Sciences (telephone Dundee 23181 ext 324). 3751(E)

POSTDOCTORAL FELLOWSHIP IN IMMUNOLOGY

Recent graduate to work on lymphocyte activation, macrophage function. Renumeration is $12,200 pa, renewable. The position is available immediately.

For details contact: Dr J Gordon, Dept of Surgery, Div. of Surgical Research, McGill University, Donner Bldg., 740 Docteur Penfield Avenue, Montreal, Quebec, Canada, H3A 1A4; telephone: 514-392-4859. W524(E)

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Brunner Straße 59,
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W535(E)

THE ROYAL SOCIETY
JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1980

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology. This award, which may be held for periods from three months to one year will in 1980 be up to £2,000, adjusted according to qualifications and place and duration of research. Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment. Applications receivable by 15 December 1979 should be made on forms obtainable from The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. 3605(F)

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Metallurgist or materials scientist for major research project on marine corrosion and fouling of North Sea oil and gas installations, financed by SRC Marine Technology Directorate. RGIT's marine corrosion research group will collaborate with University of Aberdeen marine biologists and zoologists and with offshore industry.

Salary range £4,910 to £5,777 with appropriate placing according to qualifications and experience.

Details from Secretary, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen, AB9 1FR. 3745(E)

UNIVERSITY OF SUSSEX
School of Mathematical and Physical Sciences

RESEARCH FELLOWSHIPS IN THEORETICAL ASTRONOMY

Applications are invited for SRC-supported posts of Research Fellow in Theoretical Astronomy, for a period of one or two years, with possible extension to a third year. The posts are on the Research Fellow 1A scale (£4,333 to £7,521 pa, under review). Appointments are likely to be made no more than half way up the scale. All posts carry USS benefits. Appointments will normally commence on 1st October, 1980.

Candidates should propose an independent research programme. Further information can be obtained from Professor R J Tayler, Astronomy Centre, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH, UK.

Applications should arrive by 12th January 1980. Applicants should ask up to three referees to send confidential reports, to reach the Astronomy Centre by the same date. 3721(E)

WORKSHOP

EMBO WORKSHOP

GENETICS AND IMMUNOBIOLOGY OF PATHOGENIC *NEISSERIA*

June 16-19, 1980, Hemavan, Sweden

Cosponsors: The Swedish Medical Research Council, Umeå university, Astra, Eli Lilly, FOA, Pharmacia

The objective of the workshop is to evaluate our current under-standing of the basic biology of pathogenic *Neisseria*, with special attention given to genetic aspects, cell surface organization and host-parasite interactions.

Main topics:

I ANTIGENIC AND BIOCHEMICAL CHARACTERIZATION OF THE *NEISSERIA* CELL SURFACE
II GONOCOCCAL AND MENINGOCOCCAL GENETICS
III HOST-PARASITE INTERACTIONS
IV PROSPECTS OF A GONOCOCCAL VACCINE

Invited speakers:

T. Buchanan (Seattle), S. Falkow (Seattle), C. Frasch (Bethesda), J. Heckels (Southampton), K. Holmes (Seattle), K. Johnston (Dallas), Z. McGee (Nashville), L. Mayer (Salt Lake City), S. Morse (Portland), R. Rosenthal (Indianapolis), F. Sparling (Chapel Hill), J. Swanson (Salt Lake City), D. R. Veale (Birmingham), F. Young (Rochester).

Organizers: D. Danielsson/S Normark

Applicants (actively engaged in the field) should submit a preliminary abstract of their poster and a brief description of their interest in the above topics. Participants will be accommodated in the congress hotel at Hemavan, situated in the northern Swedish mountains.

Total costs: including registration fee, travel from Umea to Hemavan, accommodation and meals 900 Swedish crowns. There are some possibilities for travelling support particularly for younger participants. It is suggested that all contributions should be published.

Applications, requests for further information and suggestion should arrive before **January 1, 1980** at:

Department of Microbiology
Unversity of Umea
S-901 87 Umea, Sweden.
Telephone: 090/10 1774.

W536(V)

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3765(L)

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Academic Year

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc. and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Bio-Inorganic Chemistry to Chemical Physics. For first year students the minimum stipend, including the summer research assistantship, is anticipated to be $8,000 per annum.

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Application forms and further particulars may be obtained from the undersigned:

Professor C.A. McDowell, Department of Chemistry, The University of British Columbia, 2075 Wesbrook Mall, Vancouver, B.C. Canada, V6T 1W5 W525(P)

IMPERIAL COLLEGE
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DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANTSHIP

A postdoctoral research assistant is required for structural investigations of the erythrocyte membrane protein spectrin, using monoclonal antibodies to study functional sites and molecular interactions. A sound background in protein chemistry and/or immunology is required, preferably with experience in monoclonal antibody techniques. The successful applicant will join an active, well equipped research group, preferably starting on January 1st 1980.

The position, supported by the MRC, is for an initial period of one year, extendable up to three. The starting salary will be £6,228 p.a. including London allowance of £740 and U.S.S. benefits.

Applications including a curriculum vitae, a full statement of research interests and experience, and the names of two referees should be sent to Dr David Shotton, Department Biochemistry, Imperial College, London SW7 2AZ England. 3769(P)

COURSES

EMBO

Advanced Laboratory Course on "Molecular Biology of Membranes"

co-sponsored by FEBS & SKMB

14-27 September 1980
Bio-Center, Basel, Switzerland

The course will include review lectures, specialized lectures and laboratory experiments. Main topics to be covered will include the physical chemistry of membranes, membrane reconstitution and analysis of membranes by biochemical, genetic and immunological methods.

Laboratory space will be available for 24 students. The course fee will include Swiss Francs 100 for registration and approximately Swiss Francs 500 for accommodation and food. Fellowships will be available. Applicants will be selected on a world-wide basis.

Application forms can be obtained from the organizers. Deadline for application is April 1, 1980. Organizers: G. Schatz and J Seelig, Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. Tel. 061/25 38 80.

W520(D)

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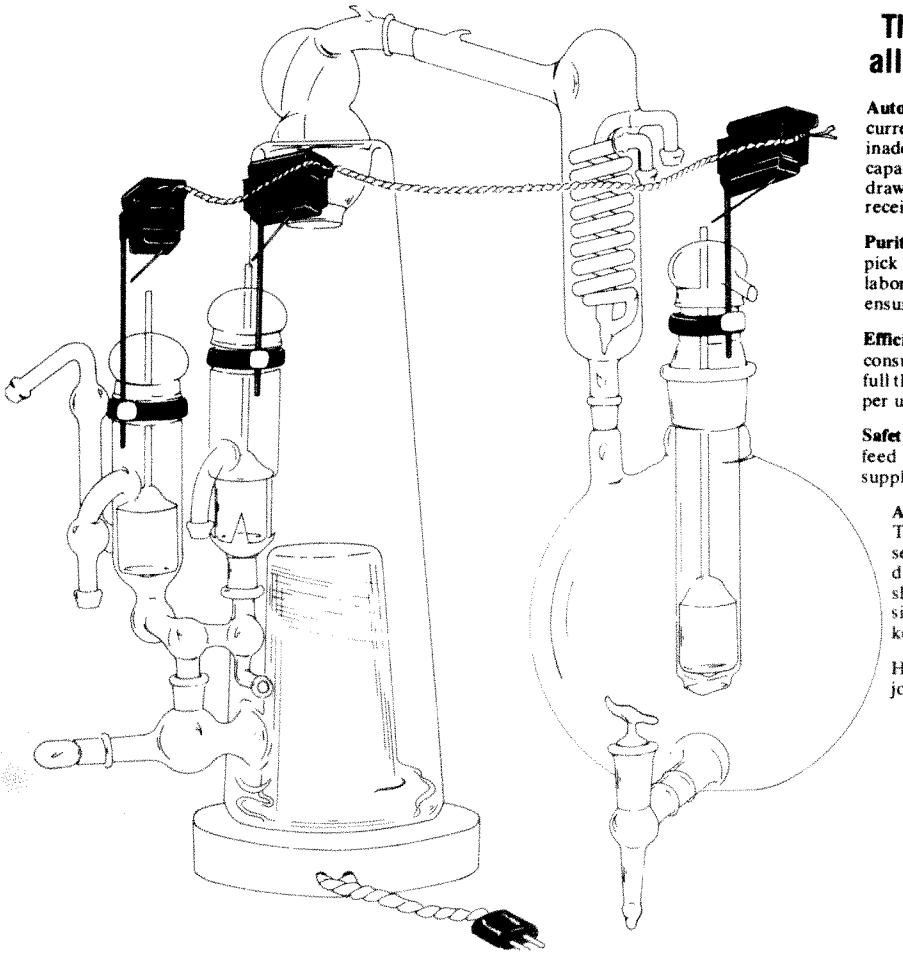
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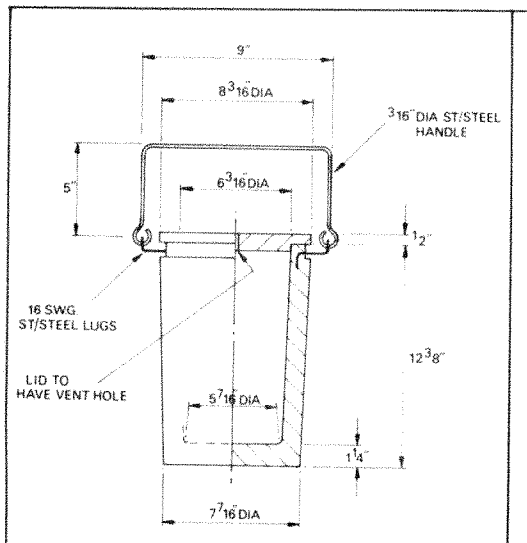
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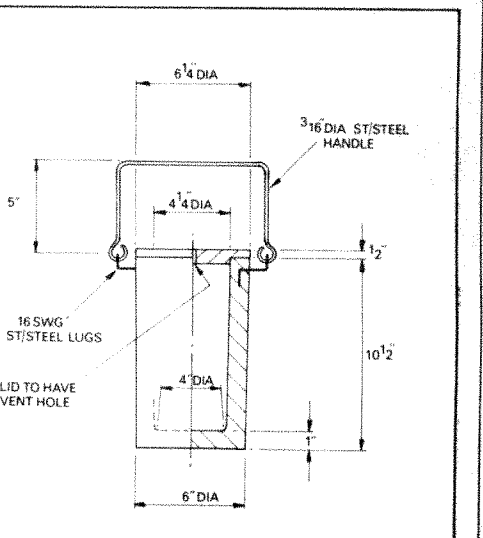
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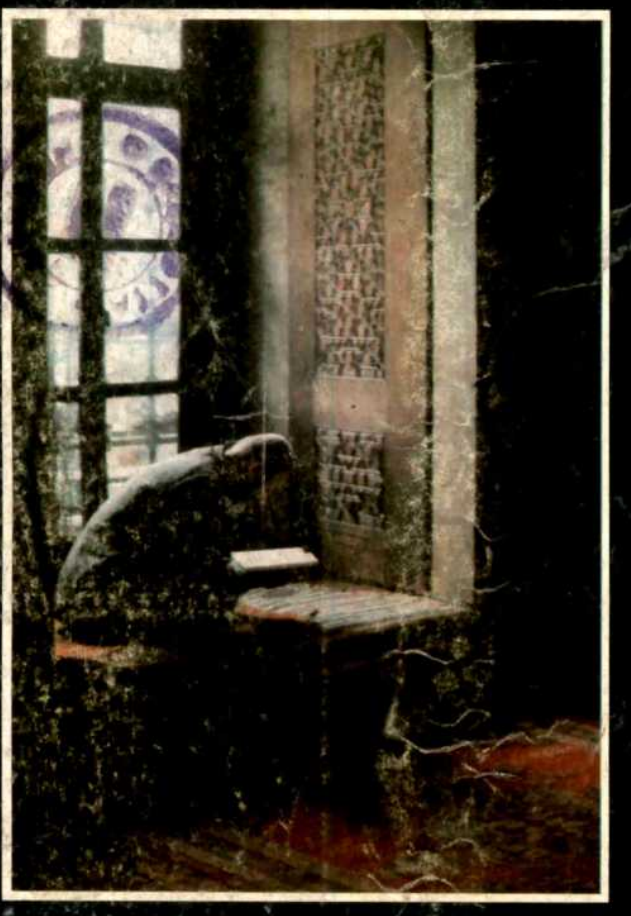
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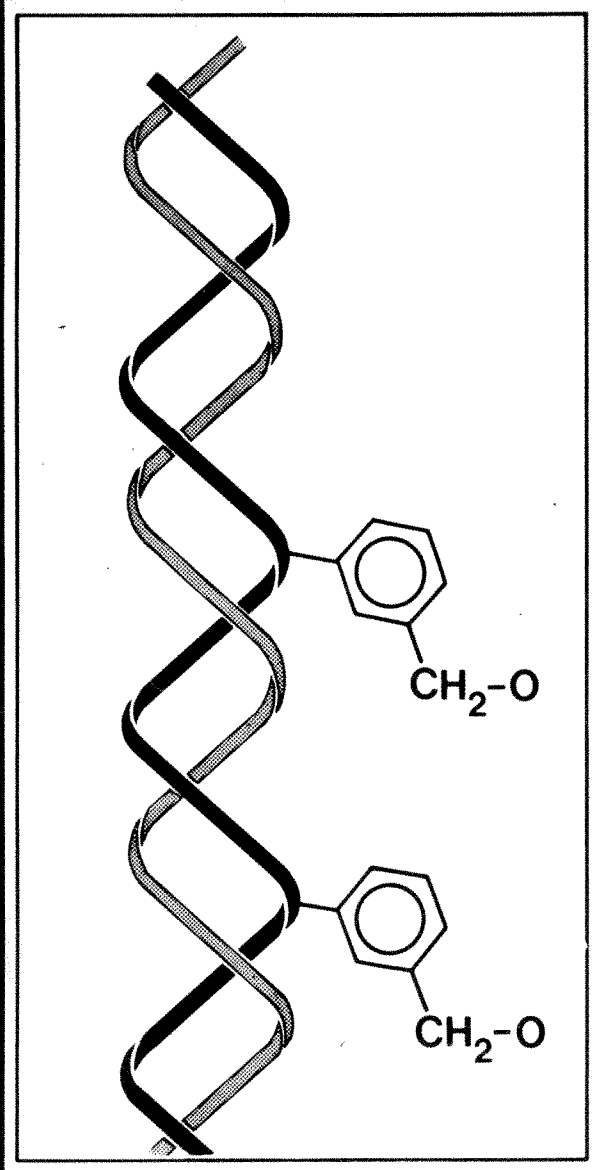
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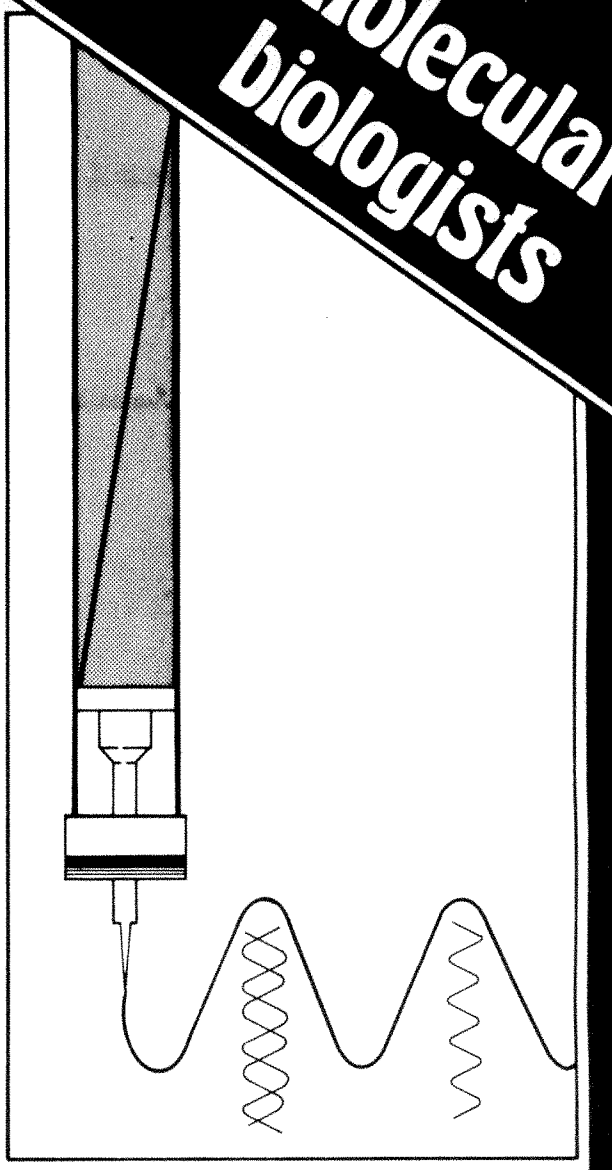
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A Turkish Moslem deep in contemplation of the *Qu'ran*, which extolls symmetry and natural beauty. Zia Sardar begins a series on Muslim science on page 354.

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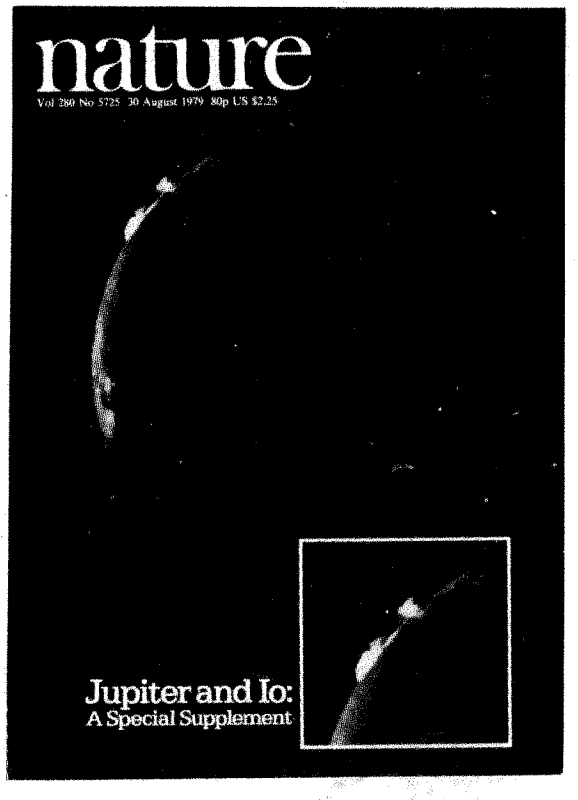
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NEW PRODUCT REVIEW

This month's new product review features oscilloscopes, laboratory computers and calculators, and image analysis equipment. See page xvii.

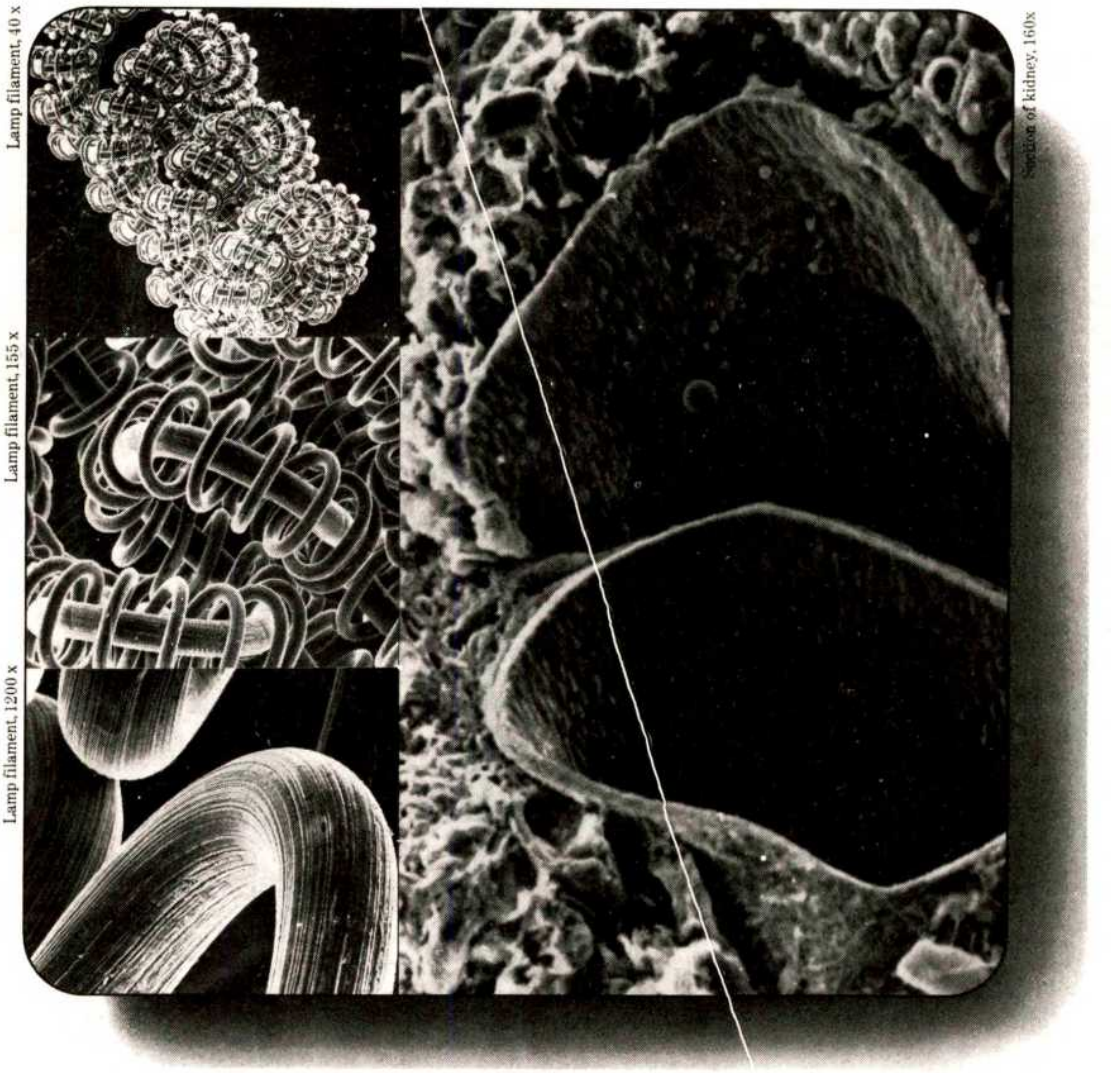
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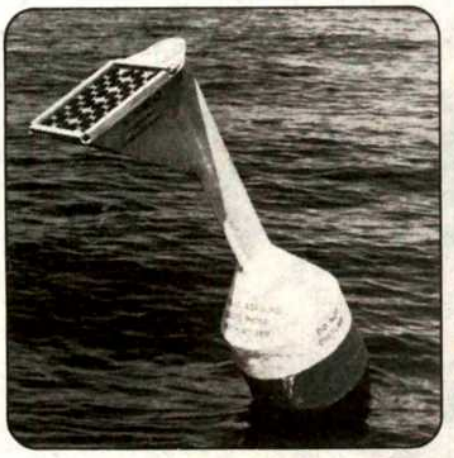
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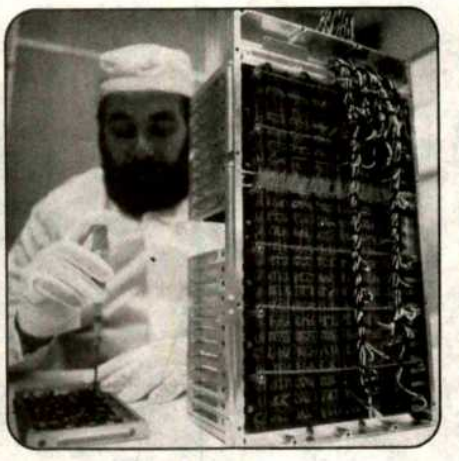
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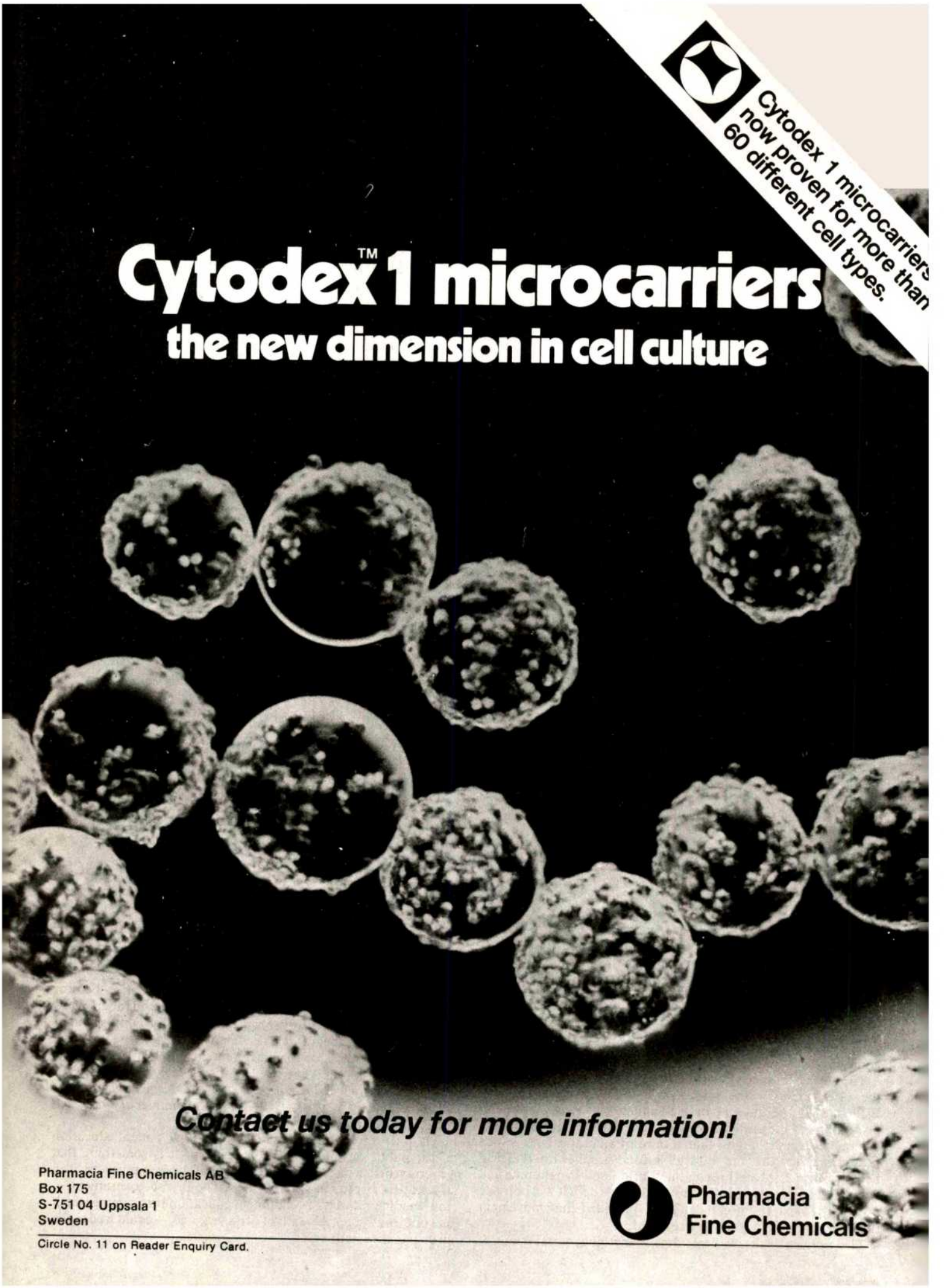
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The Cambodian picture becomes clearer

In the past few weeks, the people of many countries have been exposed to the plight of Cambodia, or Kampuchea, through television programmes, through dramatic gestures, such as Mrs Carter's visit to Thailand, and through the widespread publicity of the relief agencies. The response has been generous; governments have pledged all manner of aid, and voluntary bodies are receiving a steady flow of funds. There is an evident need for the care of many thousands of Khmer refugees in Thailand. But as more is learnt about the situation within Cambodia, it is emerging that the need is not so much for immediate, massive emergency aid to relieve a national famine (for which there is little evidence), but for steady aid over the medium and long term to bring a once flourishing country back to health.

Observers who have recently been in Cambodia, or who have worked amongst the refugees in Thailand, are now beginning to bring back a more comprehensive picture, and this is augmented by satellite photographs of areas under cultivation. As far as food is concerned, it is likely that a substantial harvest was available at the end of 1978. Most of the country has one harvest per year, so food resources under normal circumstances should have been fairly adequate for most of 1979. Of course the circumstances are not normal and much of the stored rice could have been lost; possibly upwards of 200,000 tonnes of food aid has been shipped in this year at the request of the government but this should be put in the context of an annual consumption of about 1 million tonnes (assuming a population of 4.5 million). Where simple indicative tests such as the measurement of mid-upper arm circumference of children have been carried out, they equally suggest moderate under-nutrition rather than starvation.

The mid-term food supply in Cambodia depends very much on the 1979 harvest, which runs from October to December. Estimates of the amount of land under cultivation after the disruptions of the past year vary greatly, but the figures that are emerging suggest that less than a third of all arable land will be productive, and that the shortfall in food supplies for 1980 will be up to 50%; pledges through the World Food Programme and from individual countries will ensure that this deficit will be made up for the next six months at least, provided that movement through ports of entry can be expedited.

The problem on the medical side is, if anything, more serious. It is reported that malaria is killing widely, and that untreated gastro-enteritis, tuberculosis and anthrax are also taking a heavy toll, although figures are proving hard to come by. During the dry season (until April) the incidence of malaria will fall, but concern is being expressed about the degree of drug-resistance being shown. Medical supplies have poured in beyond the capacity of local staff to use them and the problem for the immediate future is the development of a proper medical infrastructure to provide health care. With the (literal) decimation of the professional classes in Cambodia, no real progress towards self-sufficiency is likely until new schools are built, new people are tained and self-confidence is re-established.

The real need for Cambodia is not to relieve a nationwide famine; it is to supply food, medical aid and transport steadily and intelligently over the coming months, and for a large scale investment both in rural and urban re-construction and in the rebuilding of the professions. This poses difficult questions for the more thoughful of those connected with relief. Film of starving children (and it cannot be denied that some children, particularly amongst the displaced, are starving), mention of widespread famine and hints of many further deaths in the coming weeks undoubtedly opens the floodgates of goodwill, but it also leaves relief agencies with strong political and emotional pressures to act on an emergency basis, even when it were better that investments were delayed until more information became available. There is a very serious dilemma here. Fund raisers know full well that tragic pictures bring in the money, but fund-spenders know equally well that the real needs are often elsewhere, or over a different time-frame, or for a different purpose. They know, for instance, that any money given now to alleviate the effects of the severe drought which has hit parts of India this year is ten times more valuable than cash that will flow in the summer of 1980 when the effects become all too visible.

The only resolution of this dilemma is slow, patient education of the public on the value of prevention. It is something that governments themselves should take on, if they see any merit at all in scientific assessments of disasters. The British government — now winding down its development education programme which had only been given a breath of life a year ago — could well make an imaginative step. □

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Sociobiology critics claim fears come true

The National Front in England and the Club d'Horloge in France draw inspiration and support from human sociobiology, its critics said in Harvard last week. **David Dickson** reports.



National Front demonstration in Essex. NF literature has drawn on sociobiology texts.

HUMAN sociobiology came full circle last Thursday, when critics from Harvard University — the theory's principal *alma mater* — argued that its recent use by right-wing groups in Europe vindicated their earlier statements about its inherent political content.

A packed audience in the university's geology lecture hall was told that events in England and France supported warnings issued four years ago, when a biological interpretation of human social behaviour was suggested by Harvard zooloist E O Wilson in his book 'Sociobiology: The New Synthesis'.

The European experience "has realised some of our worst fears" said Dr Jonathan Beckwith, a geneticist at the Harvard Medical School. In England, for example, the extreme right-wing National Front party had used statements by Wilson and others in its literature to justify racism as a strategy for ensuring genetic survival. Similarly in Paris, right-wing intellectuals had used the writings of sociobiologists to defend claims about the inevitability of human hierarchies, said Beckwith.

In the US, sociobiology had appealed less to extremists than to those endeavouring to preserve the status quo against the claims of feminists or minority groups. But with the US entering "a new period of racial strife" extra caution was needed. "The European experience shows that groups who have used these ideas consider them to be powerful tools for rationalising their policies", said Dr Beckwith.

The Harvard meeting was organised by the sociobiology study group of Boston's Science for the People. Members of the group emphasised that they were not branding all those who wrote about sociobiology as extremists — but were drawing attention to the potentially dangerous connections between ideas expressed by scientists and the social and political climate in which they were propagated.

Thus two speakers warned that biological ideas about human nature had underpinned the practices of Nazis in Germany prior to and during the Second World War. Dr Stephen Chorover of the Massachusetts Institute of Technology said that the roots of Nazi extermination policies could be found in ideas about 'social biology and racial hygiene' which arose in Germany in 1904, when a concern for the health of the individual factory worker evolved into a generalised concern to protect the public health against external threats.

Ideas about the 'naturalness' of social roles also permitted the Nazis to extol the role of women as housewives subservient to men, said Freda Salzman, a physicist from the University of Massachusetts.

Dr Richard Lewontin, Professor of Biology at Harvard, defended the scientific attack on human sociobiology. There was no evidence, he said, of a single human behavioural characteristic caused by genetic variability, as sociobiologists claimed. Labelling all critics of sociobiology as 'reds' was a "last refuge" which ignored scientific critics holding a variety of political views.

"The problem for the ideologues in our society is that they are unable to offer a reasonable excuse for continued inequality in a society which is claimed to be democratic" he said. Hence the temptation to label social inequalities as inevitable.

Dr Beckwith produced an article 'sociobiology; the instinct in our genes', which appeared earlier this year in the National Front publication *Spearhead*. The article says that scientists from the early eugenicists to modern sociobiologists have demonstrated that social differences have a biological basis, thus showing up "the genetic basis or racial awareness, discrimination and solidarity".

Similarly in Paris, said Dr Beckwith, right-wing intellectuals had recently gained wide publicity for their statements that hereditary differences posed insurmountable barriers to social equality. Members of groups such as the Club de l'Horloge used sociobiological arguments to support their claim that people with superior genes should become the leaders of society.

In both countries, the rise of right-wing groups resulted from economic difficulties. So far in the US, the use of sociobiological ideas had focused primarily on sex roles. Newspaper articles said that men would always play a dominant role in politics because of their 'natural aggression'. Sociobiological influences were growing in school text books as an explanation of sex roles. "Exposing sociobiology for what it is is an important part of the battle against racism and sexism in our society" said Dr Beckwith.

No sociobiologist had been asked to respond. But speaking from the floor, Dr Irven Devore, Professor of Anthropology at Harvard, said that sociobiology was not being treated fairly by its critics — and that it was necessary to separate the scientific content from some of the claims being made for it.

Dr Devore agreed that the world-wide swing to the right was an "unhealthy movement", but denied that sociobiology should take any of the blame. "Historically it is unfortunate that this movement has taken place at a time when ideas about 'inclusive fitness' have emerged in biology that have revolutionised the study of animal behaviour. It is a question of whether these ideas are totally a product of that political/ideological shift or not. I do not think they are."

The critics remained firm. Dr Beckwith emphasised that the political uses of human sociobiology were inherent in its construction, not merely abuses of a neutral theory. Dr Lewontin said that the Chinese might refer to it in terms of the three vulgarities: "vulgar Darwinism, vulgar Mendelism, and vulgar reductionism." □



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Oceanographers fear greater federal involvement

THE increasing activities of mission-oriented federal agencies in oceanographic research threatens to undermine a creative tension between government, industry and universities, according to a report published by a panel of the National Academy of Sciences.

The report also warns that a desire for "useful" research, coupled with possible research restrictions in foreign territorial waters, is shifting the emphasis from oceanography as a science searching for principles and understanding to oceanography as a tool with which to accomplish practical goals.

The report was prepared by an *ad hoc* committee established by the Academy's National Research Council to study the geological and geophysical research needs and problems of the continental margins. The chairman was Dr Alber W Bally of the Shell Oil Company.

Stating that a substantial increase in funding for research on domestic continental margins could produce considerable benefits in terms of both scientific knowledge and information about mineral resources, the committee says that it is disturbed by recent trends in the distribution of responsibilities between government, universities and industry.

"Overall federal money for oceanographic research has increased substantially in recent years, but most new funds have gone to mission-oriented government agencies. Funding for basic oceanographic research done by scientists in academic institutions has barely kept up with inflation", the report says.

It suggests that government should concentrate primarily on research related to its function of governing and regulating the use of public resources — and that a considerable amount of future research effort should be delegated to universities.

The report also expresses concern about the possible impact of restrictions emerging from the Law of the Sea Conference, now under negotiation by the United Nations, under which scientists would require permission to conduct research within 200 miles of a country's coastline.

Given these restrictions, as well as the importance of scientific knowledge as a basis for resource exploitation, the report argues that "the time is ripe" to concentrate more research on domestic continental margins.

"Science — and particularly oceanography — is international. However we believe that an effort with increased emphasis on national concerns is needed. We are recommending an increase in the momentum of solid-earth science research in our own back yard."

Commenting on the likely restrictions on international research, the report points out the irony that in recent years, pressure to demonstrate the social relevance of scientific research has caused university scientists to submit proposals containing rhetoric extolling the potential social impact of their work.

"Because of the emphasis placed on justifying research as having significance for resource evaluation, great strain may be placed on the credibility of the researcher if he proposes to do similar work on foreign margins, claiming it to be 'pure' scientific research" it says.

The report suggests that a strong academic domestic research programme on US continental margins might help to convince foreign sceptics that US academic institutions "are not implicitly interested in prospecting, that their interest is to contribute to the understanding of principles related to resources and to the environment." □

WHO looks at bacteria for vector control

THE potential of certain bacteria, notably strains of *Bacillus thuringensis*, for the control of insect vectors of tropical diseases is being actively examined by the World Health Organisation, in collaboration with a number of laboratories on both sides of the Atlantic. For many years, strains of *B. thuringensis* have been marketed by a number of pesticide manufacturers in commercial formulation for use against the larvae of various lepidopterous pests, but it is only recently that the potential of the serotype H.14 (also known as *B. thuringensis israeliensis*) has been fully appreciated. This strain is now known to be extremely lethal for the larvae of certain Diptera, including mosquitoes of the genera *Aedes* and *Culex* which are vectors of malaria and filariasis respectively (although somewhat less so, apparently, against *Anopheles*). Now WHO sees the possibility of using it to control the blackfly, *Simulium damnosum*, the vector of onchocerciasis (river blindness).

On the commercial side, development is already going ahead, notably on the part of the Franco-Belgian firm Bellon-Biochem. They are working in close collaboration with the Pasteur Institute, where Dr Huguette de Barjac leads a team in this field. The first results from serotype H.14 formulations were so encouraging that Biochem decided to go straight to field testing, thus leap-frogging the lengthy procedure in a way that WHO itself could never do. The results against blackfly larvae in the Ivory Coast have so far shown very high toxicity rates, with, a report states, "nearly total short-term innocuity against the associated non-target fauna".

The nature of the new material, however, has raised problems in its large-scale application that have yet to be resolved. The larvicidal action, it appears, is due to crystalline toxic compounds present on the outer coating of the bacterial spore. These operate in fact as a chemical insecticide. Unfortunately they have a high specific density, tending to sink to the bottom in water, and one problem is therefore to find a formulation that will keep the toxic materials afloat long enough for them to reach the target larvae downstream of the point of application. This in turn raises difficulties in attaining the concentration necessary to achieve the experimentally predicted results.

It is therefore necessary to put the maximum amount of crystal toxin into the primary powder used as a basis for the actual pesticide, and then to formulate the latter in such a way as to give the desired residual effect under normal field operating conditions. From the commercial point of veiw, the development of such a formulation could probably enable the manufacturer to patent his material, a matter of great importance in the highly competitive pesticide industry. Moreover, any such formulation must also pass the stringent requirements of most governments with regard to safety against mammals. This aspect is also being examined in a number of laboratories. However, with government support in several countries — notably France, the USSR and Switzerland — it looks as though the new materials may reach a stage in the quite near future where mass field trials can be undertaken under WHO auspices.

The current resurgence of malaria, with increased resistance to normal insecticides as well as to therapeutics, and the pressure to find alternatives to such materials as DDT, will certainly encourage research in this field. Quite apart from its enormous potential for use against the vectors of malaria and other mosquito-borne disease, the new material could provide a valuable stand-by alternative to the pesticide Abate. The huge onchocerciasis control programme in the Volta Basin in West Africa at present depends entirely on this one pesticide for blackfly control.

Peter Collins.

France seeks bioengineers

France this year needs 30-40 biotechnologists familiar with such things as the purification of proteins, fermentation, and cell cultures. Next year there will be a need for 60-80 and the year after 120-160, according to a report reviewed last week by *le Monde*. The report is part of a massive work 'Sciences de la vie et société' prepared by Francois Jacob and available for consultation in Paris. □

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European Science Foundation expands 'additional' activities

THE European Science Foundation, which over five years has established the role of 'stimulant' for European cooperation in basic research, is increasing its emphasis on 'additional', directly funded research. Approximately half of ESF's 10 million FF expenditure is now devoted to such work, and the proportion is increasing.

One example is a proposal for work in taxonomy, which the ESF believes is neglected in Europe — to the extent that assessment of environmental impacts (of SO_2 for example) are hindered. A 'European Floristic, Taxonomic, and Biosystematic Documentation System' should thus be established, and the work required would become an 'additional activity' of the ESF. This means that the 46 national research councils which are members of the ESF will be asked if they would like to contribute to the activity as an addition to their membership subscription to the Foundation.

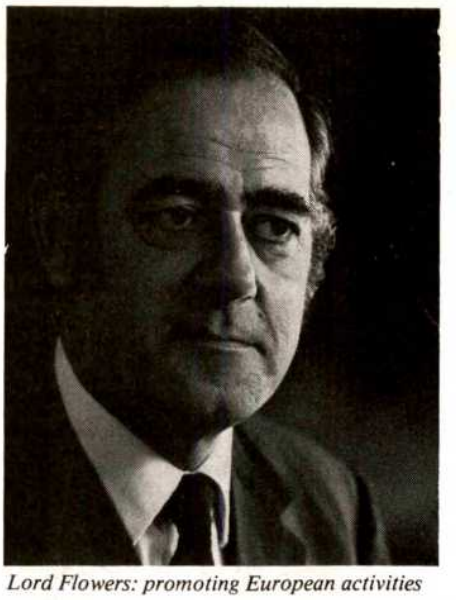
Lord Flowers, in his presidential introduction to the 1979 annual report (to be published shortly) says: "It is through the additional activities that our work as a foundation will stand or fall". But he adds "we are conscious that each new activity adds to the financial burden of those of our members who participate in them".

For some additional activities, however, funds have been made available from outside sources such as the Leverhulme Trust and the Commission of the European Communities (which is supporting a study on the mobility and employment of scientists).

"Participation in the additional activities represents on the part of our member organisations a conscious and deliberate intention to give a certain priority to research which is conducted at a European level" writes Flowers.

The increasing importance of additional activities, which was hardly foreseen when the ESF was set up, may however lead to some difficulties among its members. They will be increasingly invited to fund projects controlled not from their own offices but from the ESF, testing their commitment to Europe. Thus, says the report, "money from an organisation in country A can and will be used to pay a scientist coming from country B, and possibly working in country C". Some members have already set up a new category in their budgets for 'European cooperation' to allow for this, says the report.

Another indication for the future of the Foundation was the agreement at this year's annual general meeting, held in Strasbourg earlier this month, that tentative approaches should be made to Soviet and East European scientific bodies to enquire if they might participate in some of the ESF's activities — without becoming official members. Lord Flowers will visit Moscow next year with this in mind.



Lord Flowers: promoting European activities

Turkey's Scientific and Technical Research Council also joined the ESF this year, and Finland, announced it will become a full member. **Robert Walgate**

European synchrotron radiation facility brings governments together

IN AN unprecedented move, the European Science Foundation is to approach governments — albeit gingerly — to discuss the construction of a $120 million European Synchrotron Radiation Facility.

Officially, the ESF will have nothing to do with governments, and preserves its independence jealously. Unofficially, the retiring secretary-general of ESF, Dr F Schneider, will in his retirement be convening an intergovernmental committee with the object of making the ESRF a reality. Thus the committee will strictly not be a part of ESF activities, although it is ESF-inspired.

Dr Schneider, who is retiring this year to be succeeded as ESF secretary-general by Belgian lawyer John Goormagtigh, complains in an unsigned introduction to this year's annual report, that the lack of access to governments hampers the Foundation. The ESF's influence compared unfavourably with that of the National Academy of Sciences in the US, says the article. "A project proposed by the NAS is brought to the government for decision. The National Aeronautics and Space Administration's space science budget is decided in one place, not fifteen capital cities."

The report recommends a new role for the ESF: "In cases where concerted action is needed, a new machinery enabling the Foundation to approach governments in the name of all its members [national research councils] and to act for them ought to be developed".

The ESF has prepared the case for the facility in two booklets produced by Yves Farge, director of the Laboratoire pour l'Utilisation des Rayonnments Electromagnétique (LURE), Orsay, and collaborators. The booklets are titled "ESRF: the feasibility study" (pp67) and "ESRF: the scientific case" (pp173) (May 1979) available from H Schmied, European Science Foundation, 1 quai Lezay-Marnésia, F-67000 Strasbourg, France. □

European Space Agency "crippled space science in Europe" says ESF report

WHEN the European Space Agency was established, funds for scientific research were confined to no more than 12-15% of the whole budget, to make room for an applications programme and the development of Ariane (ESA's own launcher, due for test flight in December) and Spacelab. "It is now apparent that this was wrong" says an independent report just published by the European Science Foundation. The chosen level of funding "seriously crippled space science in Europe", leaving European space scientists with only one sixth of the resources available to US scientists.

"Launches of ESA satellites are so infrequent" claims the report "that each hardware-building institute in Europe has approximately two chances in ten years" of participating in an ESA payload. "This is utterly inadequate, because the programme is so small that new talent is not likely to be attracted". Some 1500-2000 scientists in the countries of ESF members are presently using the results of space investigations, the report estimates.

Financial pressures have also limited ESA's collaborations with the National Aeronautics and Space Administration; ESA's contribution to the space telescope, for example, is only 15%. Europe should aim to match the US and the USSR in space science, says the report. An annual ESA launch rate of one satellite fully instrumented by European groups, and "additional spacelab flight opportunities" will be required to keep European groups above "the critical threshold of efficiency".

The report recommends that ESA increase its mandatory scientific budget. But this requires unanimous approval by all ESA member states, and is therefore unlikely. Optional programmes could augment the mandatory budget, however, because they allow funding to be more flexible, says the report. "Space Science in Europe" (pp66) available from H Schmied, ESF, 1 quai Lezay-Marnésia, F-6700 Strasbourg, France. □

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"Hidden cuts" will damage UK medical research

THE Medical Research Council, like other UK research councils, has had to face "unprecedented" and immediate cuts in its current budget, as a result of the present British government's policy of reducing public expenditure. But although these amounted, in the MRC's case, to a £500,000 reduction in direct funding from the Department of Education and Science, "hidden cuts" would hit the MRC harder, said its secretary Professor J L Gowans at a press conference last week.

These were losses through having to meet pay increases from within a falling budget, through having to pay higher value added tax (the rate has been increased from 8% to 15%). And the tropical disease research programme of the MRC has been badly hit by the reduction in budget of the Ministry of Overseas Development, when it was absorbed into the Foreign Office to become the Overseas Development Administration. The total cuts amounted to some £2.1 million, or 3.5% of the MRC budget, said Professor Gowans.

Furthermore there was no firm information available to the MRC on which it might make plans for 1980-1 and beyond (though Professor Gowans expected that it would sustain further cuts). This caused great difficulties "as the shortest period for which we have to plan funding is three years, the duration of a research grant".

"We need a stable budget both from the DES side, and from the universities." The dual support system, whereby the universities themselves provided the basic facilities for a laboratory — building, stores, technicians and so on — was creaking.

Universities were also sustaining cuts, and in endeavouring to maintain teaching were leaving more and more of the basics of laboratory funding to the research councils. The system was "creaking under the strain".

"If in addition to the cuts we took this year, there are cuts of any magnitude in successive years, then damage will be done to medical research in Britain . . . There is a danger that high class new applications for research will go unfunded."

However, despite the cuts the MRC managed to set up three new research units in the past year: a molecular haematology unit in Oxford, one for neuroendocrinology in Newcastle, and an environmental epidemiology unit in Southampton.

The council's annual report for 1978-9, just published, pays attention to the jobs problem facing medical researchers. The MRC, which employs 991 clinical and non-clinical research scientists, "is constantly aware of the need to keep under review its career structures in relation both to the interests of the individuals themselves and the advancement of science" says the report. The report offers no panaceas.

●The Advisory Board to the Research Councils, which coordinates the direct funding of the councils through the Department of Education and Science, is consulting with the Treasury and the National Research Development Corporation to see if there can be any financial return to the Councils from patents arising from research council funded work. At present all benefits from these are retained by the NRDC, a large fraction of whose income comes from patents on the 'cephalosporin' antibiotics discovered under MRC funding. The talks, however, are progressing very slowly.

Robert Walgate

Hubert Curien to head ESF in 1981

PROFESSOR Hubert Curien, 55-year-old President of the French Centre National d'Etudes Spatiale, is to succeed Lord Flowers as President of the European Science Foundation in 1981. Flowers, now Rector of the Imperial College of Science and Technology, London, was founder-President of the ESF and will have served two three-year terms when Curien takes over.

Professor Curien has played a prominent role in science politics since 1966, when he became scientific director of the Centre National de la Recherche Scientifique, the body which funds much of France's basic research. "A laboratory is not a laboratory in France without some CNRS staff" says Curien. In 1969 he became its Director-General. In 1973 he was délégué general to the Délégation Générale à la Recherche Scientifique et Technique, and since 1976 he has been President of the CNES. □

Commons debates animal experiments Bill

TWO separate bills to control the use of animals in scientific experiments are currently being considered by the UK Parliament: Lord Halsbury's Animal Protection Bill (Nature, 16 August page 534) which successfully received its second reading in the House of Lords last month, and Mr Peter Fry's Protection of Animals Bill which was read for a second time in the House of Commons last week.

Both bills are seeking to up-date existing legislation which is based on the 1876 Cruelty to Animals Act. "Whole new procedures have been developed, which I understand are totally outside the Act's jurisdiction", said Peter Fry during the opening speech to last week's commons debate. For example, the transplant of tumours from one animals to another and the implanting and developing of antibodies within animals, he said, were procedures now in use which were not covered by the Act. New legislation was needed which would incorporate these and other procedures developed over the past 103 years.

Peter Fry's Bill goes further than Lord Halsbury's in several respects. In particular, it lays greater emphasis on the need to reduce the number of animals in experiments by controlling the type of research that is done. Like Lord Halsbury's Bill, it recommends that alternatives to animal experiments should be used wherever they are available but it also emphasises the need to eliminate repetition of experiments.

One of the more controversial clauses of the Bill, however, states that "operative procedures" will only be allowed for "the advancement of biological science" in a way calculated to save or prolong human or animal life or relieve suffering. Is it possible to decide in advance what research will lead to such progress asked Tam Dalyell, who cited the discovery of enkephalin as an example of research with animals of far reaching significance which could not have been anticipated. Mr Fry accepted the point, which had also been made to him by the Medical and Agricultural Research Councils, and suggested that the relevant clause be amended "to satisfy that kind of objection".

Mr Fry's Bill also differs substantially from Lord Halsbury's over the granting of licences to experiment with animals. It recommends four different grades of licence covering all types of procedure. Grade 1 licences, explained Mr Fry, would be for the simplest procedures, such as giving injections. A Grade 2 licence would be the equivalent of the single licence now existing under the current legislation. It would allow surgical procedures under anaesthesia from which the animal is not allowed to recover. A Grade 3 licence would cover surgical operations where the animal is allowed to recover afterwards provided that it is suitably anaesthetised throughout the entire procedure. And a Grade 4 licence would be granted for "research in which pain is inevitable as part of the experiments". Mr Fry expects that this latter category will raise the most objections from the antivivisectionists.

The Bill had a successful second reading and now goes forward to the committee stage. However, Mr Timothy Raison, Minister of State, Home Office, reminded MPs that a European Convention on animal experiments was currently being drawn up and that it would be wise to wait for that before passing legislation. The government, he said, could "not regard the Bill as it stood as an acceptable way of dealing with issues of a fundamental natures concerned with animal experimentation". Nevertheless, it would not stifle the debate by opposing the Bill at this stage.

Judy Redfearn

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news in brief

Academics call for removal of Boston University President: Scientists and academics at MIT and other institutions in the Boston-Cambridge area have called for the removal of John Silber as President of Boston University. A petition circulated by Dr Salvatore Luria, Nobel laureate, and Professor Emeritus of biology at MIT, condemns Silber's administration of the university as "notorious in the academic community and the nation at large."

The current calls for censure, described by the petition as "the latest in a pattern of violations of civil liberties", have arisen from the threat by Silber to dismiss or suspend five tenured professors who had, at the beginning of September, expressed their support for striking clerical workers at Boston University. In order not to cross picket lines, the five gave their lectures in places other than the assigned class-rooms. This, said Silber, constituted a breach of contract.

The petition was launched on 5 November. Within ten days, Silber was saying that the five professors could avoid disciplinary action if they submitted a written apology. He denied that the university had ever "threatened" dismissal or suspension although these options were not, he said, "ruled out". Some milder form of sanction, such as reprimand or docking of pay was, however, possible.

USSR has no oil crisis: A new Swedish report, sharply disagreeing with current CIA estimates, claims that Soviet oil reserves are 100% larger than current Western estimates allow. The report, by Petro Studies, an independent Swedish oil research firm specialising in analysing Soviet oil and gas industries, contradicts recent conclusions made by other Western analysts. Using a systematic literature search of all Soviet information released over the past 20 years, the report says that the "large underestimation of the USSR reserves by Western analysts requires that the world's oil reserves must be revised upwards by an amount equalling the combined proved reserves of the US, Canada and Mexico." The report claims that the USSR has been the world's leading producer since 1974 exceeding the US by a factor of four and Saudi Arabia by 25% and that it will increase its oil exports to the West in order to earn hard currency. "There is no danger at all that the USSR will become a net importer of oil in the next ten years at least and it will not compete with other nations in the purchase of OPEC oil." *Soviet Proved Oil Reserves* 1946-1980. PetroStudies Co. Sjöblads väg 27, S-21370 Malmö, Sweden.

UK nuclear information restricted since Three Mile Island: An official internal memorandum advocating tight control over the amount of nuclear reactor information to be allowed to reach the public was circulated among high officials of the Central Electricity Generating Board last April shortly after the Three Mile Island accident. The memorandum, leaked to the *Guardian* on 19 November, was signed by Mr Roy Matthews, the CEGB's director of health and safety, and advocates a two step process for dealing with telephone calls from the "nuclear opposition". Step one is to refuse to provide the information and step two, in the event that the caller persists, is to request a written question which "then can be considered centrally and if it is agreed that answers should be given then we will have to insure that this is done on a consistent basis."

Windscale widow awarded £67,000: The widow of a former worker at British Nuclear Fuels Ltd's Widscale nuclear plant was awarded £67,000 in a Crown Court settlement last week. The case was brought by the General and Municipal Workers Union on behalf of Mrs Bridget McAreavey after her husband, Mr Malcolm Pattinson died of myeloid leukaemia at the age of 36. Pattinson worked as a process worker and general worker at Windscale from 1957 to 1970. He died on 28 May 1971. The case was the first in the UK in which a co mpany has admitted liability for radiation caused injury or death. (In a similar case in 1976 British Nuclear Fuels agreed to an out of court settlement of £22,500 to the widow of Jonathan Troughton and £8,000 to the widow of Harry King,both of whom died of leukaemia after working at Windscale.) An official BNFL statement said that the "settlement has been reached on the particular facts and issues in this case and should not be considered prejudicial for any future claim, pending claim, or past claim." David Gee, national health and safety officer for the GMWU was "relieved" at the action but was "not pleased" at the length of time it took to reach a decision. "These court room scenes are quite stressful and we have pressed BNFL for an automatic compensation scheme since the Troughton and King settlement in 1976. BNFL agreed to consider this but there has been no response so far. We have 30 workers at Windscale who have received maximum permissable lifetime doses and we need a protection of earnings scheme for them. Someone who is made redundant at 35-40 has a long way to go and their level of skilled wages must be protected."

ESA-NASA to launch Halley-Tempel 2 comet mission: The European and US space agencies, NASA and ESA, are seeking experimental proposals for a planned mission to two comets, Halley's and Tempel 2. The mission has been timed to take advantage of the once every 76 year appearance of Halley's comet during the winter of 1985-1986 and will last four years. NASA will be responsible for the launching and mission operations and ESA will be responsible for the Halley comet probe system.

US Army seeks new funding for nerve gas weapons: The US Army is again seeking funds for a facility to produce nerve gas weapons, halted since the early 1970s following widespread protest against the use of chemical weapons in Vietnam. Last year President Carter rejected a request from the Army to start construction of such a facility, which would produce so-called binary shells containing two separate nonlethal chemicals which combine to form a nerve gas after the shell has been fired. A report in the *New York Times* last week stated that the Army had received "tentative approval" for its request, which it has resubmitted for inclusion in the 1981 budget. However, the report also says that administration officials are split over whether the decision to proceed with the production of nerve gas weapons would alarm other member countries of the North Atlantic Treaty Organisation, where such weapons are most likely to be used in combat.

Although President Carter turned down the request last year, his budget proposal included funds to continue development work on the weapons.

Johns Hopkins gets space telescope institute: A group of US universities responsible for running a number of major US research facilities, has proposed that the Space Telescope Science Institute planned by NASA should be located at Johns Hopkins University in Baltimore, Maryland. The recommendation was made last week by a board of directors of the Association of Universities for Research in Astronomy (AURA) which is preparing to submit plans to NASA for an institute to direct space telescope research on the university's Homewood campus. The 2.5m diameter telescope is scheduled to be launched from the space shuttle in late 1983 and to operate for at least 20 years. AURA selected Johns Hopkins after examining offers to host the institute from seven separate universities.

Weiss appointed director of Institute of Cancer Research: Robin Weiss, aged 39, presently head of the Laboratory of Viral Oncology at the Imperial Cancer Research Fund, has been appointed Director of the Institute of Cancer Research in London. The ICR is a major European cancer research organisation with a staff of 450 and an annual budget of £5 million. Weiss' appointment is seen as a move by ICR to strengthen its basic research approach to cancer research.

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East Germany may debate pollution

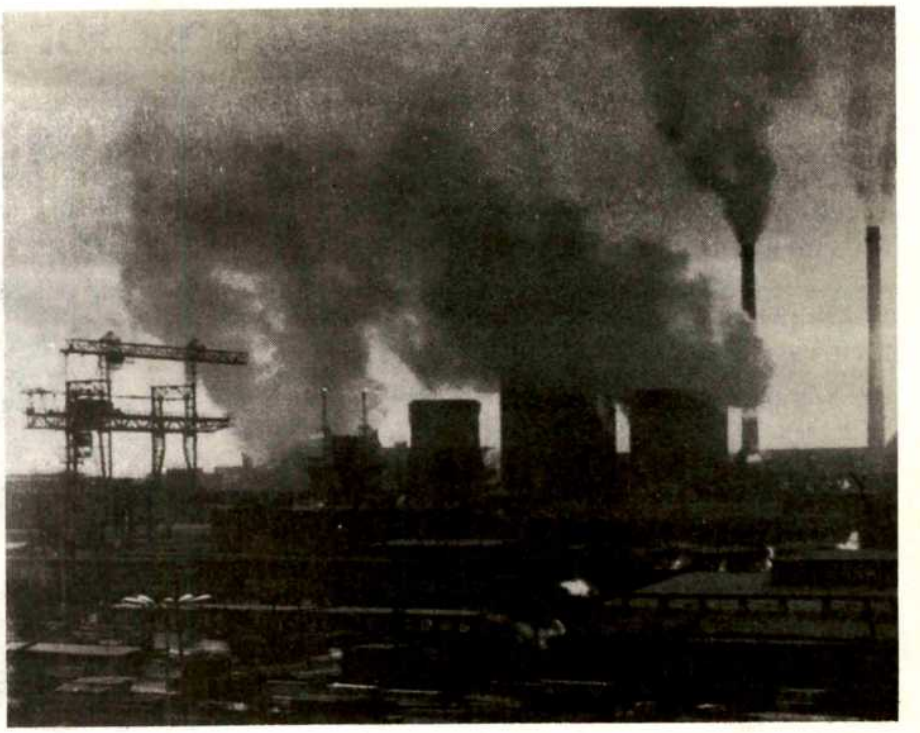
LAST week, the Mecklenberg Synod of the Evangelical Lutheran Church issued a call for public discussion on the dangers of nuclear energy — the first time that the issue has been raised in the GDR. The church in East Germany is not often outspoken on matters outside its immediate concern, and the Mecklenberg proposal for talks between politicians, scientists and churchmen on the nuclear issue is unlikely to receive unqualified official approval.

Judging from the listed "priorities" for science and technology in the current five year plan, environmental issues are not a prime concern of the East German planners, except perhaps as regards water resources which are extremely scanty (2,100 m^3/year per head of the population, according to 1970 Comecon figures, as against the Soviet Union with 19,600 m^3/year, Bulgaria with 24,000 m^3/year, and Hungary (12,000 m^3/year per head).

Conservation, to the East German planners, seems primarily to mean energy saving — as exemplified in last week's address of State Secretary Ziergiebel to the "Urania" society for the dissemination of scientific knowledge. The future problems of the energy industry are enormous and are by no means easy to solve, he said. Energy saving, in the present world situation, is "a principle of socialist economic management", which, by the use of effective technologies, can effect, as in the case of the Leuna combine, "a million-fold saving".

The Magdeburg appeal, however, seems to reflect a wider concern with environmental issues among the East German public at large. The GDR is a notoriously difficult country in which to gauge public opinion — unauthorized communication of information to foreign journalists can merit a 12-year jail sentence. However, a recent "exchange of letters" in the prestigious philosophical journal *Sinn und Form* may well serve as a pointer.





'Walter Ubricht' chemical plant, Halle, East Germany: effluents as well as profits

In the first of the letters, Gunter Kunert, a well-known writer, addresses the editor on the dangers inherent in modern technology. He ironically dismisses the "class-standpoint" that science and technology in the hands of "progressive forces" never have the same negative consequences as occur under capitalism". To take but one example, in "industrialized agriculture", "we have a metaphorical tiger by the tail", yet, in spite of the risk of poisoning, "agriculture will get nowhere without insecticides and pesticides", and to abandon them would result in world famine. Quickly he runs through a "catalogue" of possible disasters more familiar to a western than East German readership: the possibility of climatic change through CO_2 buildup and the ensuing melting of the polar ice-caps, destruction of the ozone layer by aerosol propellants, the hazards of nuclear energy — in the GDR, he said, all these dangers inherent in the development of civilization are put out of mind. DDT is banned, but new substances, whose effects are only vaguely known, are spread on the fields. No one is concerned with long-term hazards, and people simply proceed on the principle that "the human race will survive — it always has so far!"

Kunert's letter is a moderate and reasoned appeal, not for the abandoning of technological advance, but simply to a greater awareness of long-term and cumulative effects. His stance on pesticides, for example, seems very similar to the attitude of Hungary's plant protection officers. To Wilhelm Girnus, Editor of *Sinn und Form*, however, Kunert's proposals seem tainted with western doomwatch. Girnus does not, of course, dismiss environmentalism altogether; in his reply, more than three times the length of the original letter, Girnus mentions, though with some doubt, work done on the smoke-pollution of pine-trees, and even waxes enthusiastic over the Soviet Union's integrated plans (which include conservation) for the region to be spanned by the new "Baikal-Amur-Mainline".

However, his major task is dialectic. From an argument which ranges from the content of socialism to the views of a "certain Herr Schicklgruber" on the vocabulary of "Jewish liberalism", the message emerges; a doomwatch mentality, always on the lookout for "black possibilities" can lead only to "manic sweeping judgements", helplessness, and moral paralysis. In the capitalist world, he says, "a kind of addiction to catastrophic experiences" is becoming rampant. While politely agreeing with Kunert that the "warnings of a serious scientist about the dangers of a new discovery must be taken seriously", in his capacity as editor of a leading philosophical journal, Girnus makes the official standpoint abundantly clear. If citizens of the GDR do want to discuss ecology, they must eschew the doomwatch edition.

Vera Rich

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A revival for Islam, a boost for science?

TODAY is the first of the fifteenth Islamic century: a century in which Islam again is becoming a potent force in world affairs. **Ziauddin Sardar,** *Nature* correspondent for the Muslim world, recently visited eight key states — Iran, Turkey, Syria, Saudi Arabia, Egypt, Pakistan, Malaysia, and Tunisia — to report on their varied attitudes to science and technology policy. He found an increasing interest in the concept of an 'Islamic Science', a socially concerned science set within the philosophy of Islam. In the first of a number of articles which will appear in *Nature* over the next few months, Sardar here reviews opinion on whether science and technology can be so 'Islamised'. Next week he will discuss thinking in Iran; after that, nuclear power in Turkey.



Oil pipelines in Saudi Arabia: Islam's new technology

THE global middle belt: from the shores of Senegal and Morocco to the Pacific Ocean and the islands of Indonesia, and north to south from the Mediterranean coast of Turkey to Somalia; almost one billion people, constituting one-third of humanity, and speaking over 50 languages; 46 sovereign states at various stages of development and with ethnic backgrounds as diverse as those of Arabs and Afghans, the Housa and the Pathans, the Chinese and the Malays, welded together in a bond that goes back fourteen hundred years to this very day; the bond of Islam; the Muslim world.

Only three decades ago, the Muslim community was a subject community. The fifties saw the independence of many Muslim states. However, they soon learned that political independence alone is not enough. To be free and yet to be dependent on the departing colonial powers for scientific expertise, technology transfer and foreign aid is not real freedom. The Muslim world is now beginning to take the view that the post-independence development strategies occidental-style have been in error. This awakening is based on a strong self-assertion of the Muslim world's Islamic identity and brotherhood, and is seen by many as a new revival of Islam.

The Muslim world is going through a deep, although turbulent, process of self-examination and self-renewal. Almost everything is placed under the microscope and investigated from the perspective and world-view of Islam. Both western consumer materialism and eastern (dialectic) materialism are dissected with fervour. Serious attempts are being made to seek and pursue Islamic alternatives.

How would this sense of revival affect science and science policy in the Muslim world?

There is little doubt that most Muslim countries with science policies are re-examining and re-evaluating those policies. Iran, for example, is taking a very deep look at its science. Syria is reorganising its science with concern for the culture and values of its society. Iraq and Algeria are going through a process of 'Arabisation'. Attempts are being made, largely due to the efforts of the Jeddah-based Organisation of Islamic Conference, to persuade Muslim countries without science policies to formulate down-to-earth policies based on their needs and requirements. An Islamic Foundation for Science, Technology and Development (IFSTD) has been set up by the Tenth Islamic Conference of Foreign Ministers, held in Fez, Morocco, in April 1979, to help and encourage the development of science in the Muslim world, draw programmes for co-operation and co-ordination of research activities amongst the Muslim countries, and help give a unified direction to science policies (see box).

The revival of Islam is also providing an impetus for science. Mohammed Ajaz al-Khattib of the University of Damascus thinks that this is a natural process. "The pursuit of science is an obligation for an Islamic society. If an Islamic society experiences an increase in the awareness of its Islamic identity, it will fulfil its obligation to pursue science with more vigour."

According to al-Khattib, for Muslim societies science is not a luxury, but a necessity. "Some 750 verses, almost one-eighth of the Qur'an, exhort the believers to study nature, reflect, make the best use of reason, and make scientific enterprise an integral part of the community life. In contrast, the legislative verses are only 250", he says.

Kalim Siddiqui, Director of the London-based Muslim Institute for Research and Planning, told *Nature*: "The new and invigorated civilization of Islam which is now re-emerging has a solid body of axioms that have stood the test of time without the help of 'reformation' or otherwise 'revised' or 'authorised' versions. The new civilization of Islam will be capable of boosting scientific activity and scientific knowledge to heights and achievements unknown to man before".

Zia Sardar is the author of 'The Future of Muslim Civilisation' published today by Croom Helm, price £10.95 (hardback) £4.95 (paper)

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IN the history of Islam, science occupies a central position. Although known to the West largely for its historical influence on the development of European scientific thought, it also had a powerful effect on the development of Muslim civilization itself. Islamic science is considered to be one of the basic ingredients that provided the drive for the "Golden Age of Islam". So it is now being realised that science is even more essential for a Muslim civilization of the future. This realisation is obviously related to the sense of revival that is sweeping through the Muslim world. There are, however, two special factors that have helped to create this scientific consciousness.

The first was the publication of Seyyed Hossein Nasr's book, *Science and Civilization in Islam* (Harvard University Press, 1968). This was the first serious attempt to examine Islamic science *within* the context of the civilization of Islam. But more than that, because Dr Nasr, an Iranian scholar now in self-exile in London, commanded attention and respect in the West, he made it possible for many a Muslim scientist, with the usual developing country background and sense of inferiority, to speak about Islamic science without feeling ashamed. His second book on the subject, *Islamic Science: An Illustrated Study* (World of Islam Festival Trust, London, 1976), took the discussion to the popular and student level. It is responsible for generating much excitement and discussion on the campuses of the universities of Muslim countries.

The Islamic Solidarity Conference on Science and Technology, sponsored by the University of Riyadh and held at Riyadh's ultra-modern King Faisal Conference Hall in March 1976, was the second catalyst (see *Nature* **260**, 663; 1976). The conference spent a great deal of its time soul-searching and discussing "Islamic ways of doing science". At the conclusion of the conference it was declared that the future of the Muslim world is closely linked to the revival of Islamic science and the operationalisation of its concepts and principles.

That was in 1976. Since then much discussion has ensued on the nature of Islamic science. At the same time, there have been a number of attempts to 'breath the spirit of Islam in natural sciences' and to 'Islamise' the teaching of science. Notable amongst these are the innovations concerning the teaching of 'Islamic technical subjects' at King Abdul Aziz University, Jeddah; various attempts at "Arabisation of science" in Syria, Iraq and Algeria; and the entire reorganisation of education and the radical approach being planned and developed to the teaching of science in Iran.

Much of the discussion on Islamic science is woolly and confused. This is only natural. In its practical form Islamic science existed in a distant past and the Muslim civilization has been out of touch with science for over four centuries. During this period the Muslim civilization degenerated to reach its nadir, was consequently colonised, and has only recently reemerged to face what are probably the biggest fundamental material changes human life has ever experienced. Furthermore, there is an acute poverty of analytical and historical literature on the nature and style of Islamic science.

Four points of view

The discussions on Islamic science centre around two basic questions: what is Islamic science? And how does it differ from the practice of conventional science? Some Muslim scientists, however, are asking an even more fundamental question: Is there such a thing as Islamic science?

Four points of view can be distinguished on these questions. The first is what we may call the traditional (western science) ethos. The second and third viewpoints maintain that Islam has a great deal to say about science, but are divided on where exactly Islam enters the picture. The fourth argues that Islamic science is something quite different from science as it is practised today.

Ali El Hili, Dean of the Faculty of Mathematics, Physics and Natural Sciences, University of Tunis, presents the traditional view of science simply and forcefully: "there is only one science: it is universal, neutral and value-free. The study of nature is impersonal and free from human values. It *has* to be that way", he says. Discussions of Islamic science worry him. "We cannot compromise the basic rationality of science with our religious concerns". And he warns: "if we compromise the basic objectivity and neutrality of science with Islamic values and ethics we will destroy the very foundations of science".

Tevfik Karabag, Secretary-General of the Scientific and Technical Research Council of Turkey, agrees with Hili. "Science is universal", he argues, "but a value-judgement may be applied in giving emphasis and pursuing a particular area of science". Thus the Muslim countries may choose not to pursue research on fermentation due to restrictions on alcohol in Islam. However, the choice of undertaking or not undertaking a particular area of research is available only in applied fields. "Basic scientific research, in all areas, must be pursued for its own sake", says Karabag. If one were to talk about Islamic science in Turkey today, he says, one is likely to be ignored and isolated by one's colleagues and labelled a "fanatic".

There is no such danger in Egypt. Fahamy Ramadan, General Secretary of the Cairo-based National Research Centre, who spends most of his free time reading the Qur'an, declares unambigiously: "science has no home, no religion, no particular bias. Everyone contributes to it, irrespective of race, creed or culture". He



Ancient Islamic Science: an astrolabe

mourns the sorry state of science in the Muslim world and is convinced that "the road to the future of science in the Islamic world lies through Arabisation". "This is the link between our past and present", he says.

Fahamy argues that science and technology will not settle in "Muslim homelands" unless Arabic becomes, for the Muslim people, the language of science. "Only through Arabic can we encourage cultivation of sciences in the Arab and Islamic countries, encourage science to become a genuine component of Arab and Islamic thought, and assimilate the love of science in the mentality and minds of our citizens", Fahamy says.

Many Muslim scientists would agree whole-heartedly with him. Indeed, serious attempts are being made to Arabise science in Iraq and Syria. But there are a large number of Muslim scientists who argue that the issue of Islamic science is deeper than simply doing and teaching science in Arabic.

Secular or Islamic approach?

The second position on Islamic science is articulated by Affifin Suhaimi, Dean of the Faculty of Science and Environmental Studies, University of Agriculture, Selangor, Malaysia. "Science is neutral", he says, "but the attitude by which we approach science can be secular or Islamic". What is the Islamic approach to science? "We recognise the limitation of human reason and human mind; and we acknowledge that knowledge is the property of God", explains Suhaimi. "This knowledge is granted to us as a trust. We can use it for the benefit of society or we can put it to destructive use. Either way, we as scientists, are responsible before God for the use of that trust", he says.

Like Hili, Suhaimi too is concerned about the discussions on Islamic science. The argument that Islamic science is

something different from western science, complains Suhaimi, is sometimes used to block the transfer of scientific knowledge from the West to Muslim countries. "Science, in its pure, value-free form, we can take from all sources", he argues. "We should take from the West as much as we can". To support his argument he quotes a tradition of the Prophet Muhammad who is reported to have said that "knowledge is like the lost camel of a Muslim. Take hold of it whenever you come across it". "Our people forget", says Suhaimi, "that Islam is within us: we put the science to Islamic or un-Islamic use. Science is Islamised in the way we practise it and utilise it", he says.

Zafar L. Sawaf, Director of Centre for Industrial Research and Development, presents a similar argument. "Islam comes not with science, but after it", he says. "To what use do we put science in our societies? This is the Islamic question". Sawaf argues that science, in the Muslim world, has to be pursued for its own sake. "But we cannot allow certain results of science to destroy our values and culture and take our societies towards excessive materialism. We must exercise discretion in the use of scientific results and in pursuing research projects we must be aware of the effect our results could have on the moral fibre of our society", he says.

For Sawaf, the use to which science is put in society must be determined by Islam. "It is a question of preserving our values and culture. That science has led industrialised societies towards an ugly materialism and an excessive consumerism, we cannot deny. We cannot let that happen in Syria", Sawaf declares. "What we need is an Islamic approach to science."

Goal-oriented science

Values and preservation of culture also figure largely in the thinking of those who take the third stand on Islamic science. This group suggests that Islamic science is more a matter of philosophy than science but at the same time, it argues that both modern science and technology are distinctively occidental. Today, throughout the world all significant science is western both in style and method, whatever the pigmentation or creed of the scientist.

Ali Kattani, Professor of Electrical Engineering at the University of Petroleum and Minerals in Dahran, Saudi Arabia, makes the point: "Science is intricately linked with ideology in its emphasis, scale of priorities, control and the direction of research, to such an extent that scientists have now become ideologues. Their craft is not neutral, but promotes a certain pattern of growth and development and a certain ideology."

According to Kattani the Muslim scientists are only apprentices of the western scientific establishment. "Most of the research that is going on in the Muslim world is taking our societies away from Islam", he says. It has linked the Muslim scientists scientifically and emotionally with the western establishment. "So we are

Foundation for science, technology and development

AN important decision concerning science and technology in the Muslim world was taken by the Tenth Conference of Foreign Ministers of Islamic States meeting in Fez, Morocco, during 8-12 May. The decision related to the establishment of the Islamic Foundation for Science, Technology and Development (IFSTD), a supranational body that will promote, coordinate and, some hope, supervise science and technology activities throughout the Muslim world.

IFSTD had been on the agenda of the Foreign Ministers Conference for at least five years; the project was first proposed to the Fifth Islamic Foreign Ministers Conference, held at Kuala Lumpur in June 1974. Since then several studies on IFSTD have been prepared; and for the last three years a Group of Experts has been meeting to draw-up plans for launching the Foundation and finalise the $50 million first phase of its expansion.

The news of the establishment of the Foundation was greeted with considerable enthusiasm throughout the Muslim world. As Ariffin Suhami of the University of Agriculture, Seradang Selangar, Malaysia, says, "the Muslim scientists have been waiting for some considerable time for an international organisation which encourages R&D activities within an Islamic framework, and which would help solve some of the current problems of the Muslim world in particular and mankind in general. At last the Islamic Conference has responded to our needs". Many Muslim scientists see IFSTD as a key instrument in bringing the Muslim scientists together. Says Waqar Husaini of Department of Civil Engineering, King Abdul Aziz University of Jeddah: "to promote cooperation and coordination in science and technology within the Muslim world *is* to strengthen the bonds of Islamic solidarity".

The greatest hopes, however, are placed on the funding function of IFSTD. Ahmad Bourani, Director of Institute de Récherche Scientifique et Technique, Tunis, sums up the feeling of many Muslim scientists. "IFSTD can easily become another Muslim organisation with a rather large bureaucracy, unless it actively seeks viable projects that are dormant because of lack of funds. Tunisian examples would be research on arid zones, water economy and olive plants. We used to get some UNESCO backing. But it seems that they have lost interest in us. Let IFSTD pick up where UNESCO left us".

The Charter of IFSTD outlines its interest: geological surveys and resources maps, energy resources, petroleum, water resources and desalination, electronics, medical research, oceanography, space research, defence technology, ecology and food and agriculture. The accent is definitely on high technology; there is conspicuous absence of alternative technologies, though solar energy gets a passing mention. A vague reference to technology assessment is made when it is stated that the Foundation will "conduct studies on the impact of utilisation of science and technology in socio-economic plans on Islamic culture and heritage".

There are, however, a number of very original goals that IFSTD hopes to pursue, the realisation of which would make a considerable contribution to science development in the Muslim world. These include the development of "appropriate (Muslim) models of science policy" and assistance to Muslim countries to adopt them; preparation of "plans for the training of scientific manpower capable of disseminating Islamic scientific culture", and "establishment, where needed, of scientific and technological institutions in (Muslim) countries, particularly technical universities in Africa and South East Asia".

IFSTD will also study and make recommendations to Muslim countries for improving the status of scientists and technicians. In particular, special care will be given to Muslim scientists working in the West; IFSTD even proposes "to set up one or more Islamic universities for science and technology in the (developed) countries".

Science policy organisations of all Muslim countries will particularly attract IFSTD's attention. The foundation will urge them to operate within an "Islamic strategy", to promote science and technology at all levels of society, and give priority to "the creation of distinctive technologies indigenous to the Islamic world". Countries which do not have national organisations responsible for science policy and planning will be urged to establish such bodies which should be affiliated to the highest authority in the country. Where it is not possible to establish such bodies, IFSTD will undertake advisory role in science planning and policy making.

The Islamic Secretariat in Jeddah, which is also the headquarters of IFSTD, is now actively looking for a director — an "eminent scientist" — to lead it. □

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working for a society that is elsewhere, in the West. Most of our work ultimately benefits the developed countries", he argues. "Because we are working within a system of science whose emphasis and priorities reflect the aspirations of an alien culture, science in the Muslim world is either in a state of struggle or in a state of continous degeneration".

Islamic science, according to Kattani, is that science which reflects the needs and aspirations of Muslim people. "Western science places man at its centre, in the position of a demi-god. Islamic science emphasises the position of man in the universe: he can rise to the level of angels or sink to unimaginable depths, but he is responsible for all his actions, and the after-effects of his action, to a Supreme Authority. Scientific activity carried out under such a realisation is an entirely different proposition. The actual content of such a science may not be radically different. But the priorities and emphasis



Abdullah Naseef: science serves goals

will certainly be different. And so will the quality and quantity of the content of this science. And finally, the use to which this science will be put will also be different", he says.

The argument is further clarified by Raghib El Naggar, Professor of Geology also at the University of Petroleum and Minerals. "The belief in a Creator makes Muslim scientists more conscious of their activities. It places their reason and curiosity under the authority of a Supreme Power to whom they are responsible for all their actions". He quotes the verse of the Qur'an that says: "whosoever kills a human being for other than manslaughter or corruption on Earth, it shall as if he has killed all mankind; and whosoever saves the life of one, it shall be as if he has saved the life of all mankind". "We, therefore", says Naggar, "cannot let our scientific curiosity run blind for if our activities result in a single death, even indirectly, we will be accountable for it".

Thus, Naggar argues, science in Islam must be a goal-orientated activity. "Islamic science", he says, "cannot be isolated from wisdom. Neither can it be isolated from values". The integration of wisdom and values in Islamic science, according to Naggar, is in no way a restriction on thinking. Rather, "it gives one's thinking a direction". He rejects the notion of science for science's sake and considers the social function of science to be more important than science itself. "Our destiny as Muslims is determined not by science *per se* but by its social function", he says.

The fourth and the final stand on Islamic science also emphasises the social function of science but argues that Islamic science has its own, unique entity that differs considerably from science as it is practised and nourished today. Western science, it is argued, draws its inspiration from the Enlightenment and its assumption are those of the *Philosophes* and the rationalist world-view. Some of its underlying assumptions are those of medieval Christianity. As such, science, as we know it today, is a product of western civilization and an embodiment of its culture, ethos and values.

The proponents of Islamic science argue that Islamic science is based on entirely different assumptions about the relationship between man and man, man and nature, universe, time and space. Because the basic axioms of Islamic science are different from those of western science, and its method of knowing is also different, it is a science with its own identity and character.

Says Abdullah Naseef, Vice Rector of King Abdul Aziz University of Jeddah, "under Islam, science is subservient to the goals of society. The goals of an Islamic society are to increase brotherhood, reduce consumption and increase spiritual awareness. A science with these goals has to be different in nature and style from science as it is practised today. Furthermore, these goals cannot be pursued by any means. They can only be pursued by those means which are permitted by Islam. Aggressive rationalism, to give an example, is considered by Islam to be anti-human. So Islamic science does not accept the tyranny of one, supreme method. We emphasise methods in conformity with the nature of enquiry. Within these parameters, Islamic science has access to reason and experience, observation and experimentation, deduction and induction but always we turn to revelation as the supreme authority. Islamic science is the most exact of sciences without being fooled into believing that the method of experimental and theoretical sciences can lead to eternal truths."

In the post-revolution Iran, there are many scientists who hold this view. And one hears these arguments time after time. Mahdi Mumkin, Deputy Minister at the newly established Ministry of National Guidance in Iran and a theoretical architect of the Iranian revolution, holds very similar views but adds that Islamic science keeps the long-term future of society in perspective. "Muslim scientists do not seek short term expedient answers to socioety's needs. The long term view is an essential element of Islamic science. The pile-up of military weapons in the US and the Soviet Union, to give an example, is totally unrelated to any concept of a viable human future", he says. Mumkin makes an even bigger claim: "the axioms of Islamic science, its concepts of man, nature, environment, and the universe, do not permit Islamic science to become part of a system of violence — and violence is defined here in very broad terms — or of a system of power and control", says Mumkins.

Waqar Husaini of the Department of Civil Engineering, King Abdul Aziz University, Jeddah, suggests that this is because "Islamic science is



Affifin Suhaimi: objective and Islamic

epistemologically and ontologically inseparable from the Islamic concept of God." He argues that under no circumstances can Islamic science pursue research "at the expense of socio-ethical indicators of spiritual development, sanctity of family life, exploitation of natural resources without due consideration or future generations, and weapons, such as nuclear, chemical and biological, which have no regard for humanity". According to Husaini, "Islamic science is a public interest science. It operates with in the parameters of the Islamic concept of *al-maslaha* (loosely translated as the "common good") and promotes, preserves and develops 'five universal principles' which constitute the common good, namely — the Islamic way of life, life itself, reason, posterity and property".

Clearly Islamic science has a strong input from the ethics and morality of Islam. It is equally clear that it is a fuzzy concept that needs a great deal of clarification. For it to become acceptable to a wide cross-section of Muslim scientists, as well as western scientists, Islamic science has to prove itself both theoretically and practically. Considering the vast array of issues, from food, water, housing, transportation, medicine to health that now confront the Muslim world, there is no shortage of problems to work on. It will be worthwhile to observe if Muslim scientists can show that Islamic science has its own unique nature and style; and live up to their declared ideals. □

correspondence

Fast reactors will be problematical and expensive

SIR,—I did not reply at once to Dr Smith's letter (23 August, p630) which criticises my article (26 July, p 270) on fast reactors as I was not sure quite how to reply. It now seems to me that the best way I can deal with the issues he has raised and his list of my supposed errors — elementary, outstanding and otherwise — is to quote other authors who have made similar points, with appropriate references so that any interested readers can check for themselves. So this letter is rather long but the issues are important in view of the continuing discussion in Britain on CFR 1.

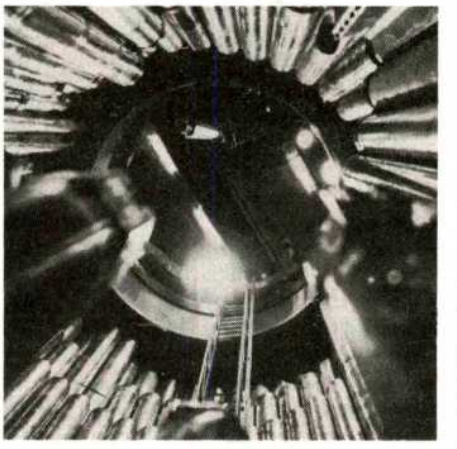
I will start with my four "outstanding errors" in physics. One is the misprint on the Doppler effect. It is remarkable that Dr Smith felt it necessary to refer to this "error" as the full version[1] of the lecture I gave at a conference in London last year has been available to him at Risley since May. My July article was simply a shortened version of the lecture, but there was no misprint in the full version.

The second "outstanding error" is my statement that a thermal reactor has less than one critical mass. If Dr Smith does not accept me as an authority on this point, perhaps he will accept Hans Bethe (Director, Theoretical Physics Division, Manhattan Project, Los Alamos 1943-46; Professor of Theoretical Physics, Cornell University since 1937; Nobel Prize for Physics 1967). Bethe[2] states in his testimony in support of nuclear power to the State Legislature of California in 1975: "The most important difference between a fast and a slow neutron reactor is that a fast reactor contains more than one critical mass. (By contrast the material in a light water reactor can never form a critical mass because the concentration of the fissile isotope ^{235}U is very low.)" The point is, of course, that a thermal reactor goes critical only in the presence of a moderator whereas a critical mass of a substance is that mass (at normal density) which goes critical by itself.

The third "outstanding error" is that "thermal reactors are designed to be in their most reactive nuclear configuration". This is indeed a possible design requirement for a thermal reactor, although not for fast reactors, and is sensible design practice for commercial thermal reactors. Compare Fauske's lecture[3] at the International Conference on Containment of FBRs at San Francisco in 1977 where he said "Unlike LWRs LMFBRs can be very sensitive to dimensional changes or relocation of core materials since the intact LMFMR core is not in its most reactive configuration". Farmer's examples (12 April, page 593) are not really relevant — *overcooling* is not a hazard for electricity-generating PWRs although it may have caused incidents in small submarine reactors.

My fourth "outstanding error" is the re-criticality scenario leading to a core-disruptive accident. A more detailed account of such accidents is given in Wilson's article[4].

So much for the "outstanding errors". Dr Smith then claims that "these errors are important in that they convey the impression that fast reactors are difficult to control, unsafe and not well understood. On the contrary . . . Their safety has been the subject of intense study for very many years and is well understood." I agree that FBR safety has been studied intensely for many years; unfortunately, however, it will have to be studied intensely for many more. This is in fact the conventional view. At the 1976 meeting in Chicago on Fast Reactor Safety, spokesmen for the UK Nuclear Installations Inspectorate stated[5] "we have indicated the seriousness with which the NII views the possibility of whole-core accidents in a thermal reactor system . . . At the present time however we believe that there are doubts about the adequacy of fault lists, about some of the physical phenomena which might play a part in an accident sequence, about structural materials behaviour, and the performance of protective systems . . . A sound programme of theoretical analysis backed up by suitable model tests is a minimum requirement." My claim that proceeding with a large fast reactor such as CFR1 with a large positive sodium void coefficient will cause even more problems is also shared by others, even by strong proponents of fast reactors such as Professor Bethe[6]. He said at Chicago[7]: "In my opinion, reactor should if possible be so designed that there is no positive void coefficient."



Inside Dounreay's PFR

If we now turn to FBR economics, the argument hinges on two basic points[8]: the comparative capital costs of FBRs and other reactor systems, for example PWRs; and the future price of uranium. Keck[9] has demonstrated in the only case study[10] available that SNR-300 will have cost 5 times as much as an equivalent PWR when it is completed. Dr Smith's comments on SNR-300 are incorrect and the interested reader should consult Keck's massive thesis[10] where a blow-by-blow account is given of how the increased costs were incurred. It may well be that the factor 5 will not be appropriate for the cost of CFR1 compared with a PWR system. Dr Smith claims that an estimate of this factor would be 1.5±0.5. I would suggest that it would be wise for the government and electricity board concerned to double this estimate to 3±1 in line with the factor of 3 assumed[11] for the proposed Clinch River breeder reactor in the US. The economist Duncan Burn who has studied the British nuclear industry for many years[12] made essentially the same point at the London conference last year[13]: "I do not doubt that following on the long development of the FBR in the UK which has culminated so far in the PFR at Dounreay we can at great cost proceed to construct one or more large 1300 MW prototypes and make a plant which will work and be safe. But it would not necessarily satisfy the economists' criteria."

Dr Smith then claims that "there is little doubt that lower fuel cycle costs will more than compensate as uranium prices increase due to pressures in the next few decades on limited supplies of uranium ore". My colleagues at the Science Policy Research Unit here are engaged in an SRC sponsored study of fuel cycle costs. Their conclusions on the future price of uranium, in line with authoritative sources in the uranium industry, are that[14] "In the longer term reserve estimates are rising rapidly, demand growth is being cut back, higher grades of ore will come on stream in the 1980s and some cost-cutting technical improvements are likely. In the probable absence of an effective cartel, this will provide strong pressure towards a lowering in the long-term real price of uranium for the remainder of this century and possibly even longer."

Dr Smith ends his letter by regretting that my article "gave such an unbalanced view of fast reactor safety". I hope that your readers will prefer the opinion of a distinguished scientist in government service who wrote to me saying "what an excellent and very fair summary".

Yours faithfully,

NORMAN DOMBEY
University of Sussex, Brighton, UK.

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Affecting the effect

SIR,—While affiantly reading page 618 in *Nature* of 25 October, I found it effascinating how an effroyable use of 'effect' effectively effeebled the meaning. Effacing the 'e' and affixing an 'a' had an efficacious effect on the affected sentence. The affeebled article's meaning now effulged.

Your affatuated reader,

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CSIRO, Epping, New South Wales, Australia.

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news and views

Anomalous ionic conductors

from Robert W. Cahn

GOOD ionic conductors such as beta-alumina, zirconia and silver oxide are the object of research in a number of laboratories on account of their potential use in high-performance batteries such as the sodium/sulphur battery. These conductors are ionically bonded crystals in which one ionic species migrates readily under the influence of an electric field; there is no electronic conduction of the metallic type.

Two recent papers in the Chinese journal *Scientia Sinica* indicate that Chinese scientists are also actively investigating at least one ionically conducting species, though curiously their interest does not seem to focus on electrical behaviour. Yang Zhen, Niu Shiwen and Cheng Yufen at the Institute of Atomic Energy of the Academia Sinica (*Scientia Sinica,* **22** (9), 1000; 1979) have studied the anomalous enhancement of neutron diffraction intensities from α-$LiIO_3$ resulting from an applied non-alternating electric field. The $\alpha LiIO_3$ crystals (made at the Institute of Physics of the Academy) are hexagonal and the field was applied along the *c* axis. The integrated intensity of the (0002) diffraction peak, measured by neutron diffraction, is sharply enhanced by a field: the factor of enhancement can reach 6 times, and saturates at a few kV cm^{-1}. α-$LiIO_3$ is polar (non-centrosymmetric) and it is thus not unexpected that equal fields of opposite polarities have effects of different magnitude on the diffracted intensity.

The applied field enhances the width of the diffracted peak as well as its height. a.c. fields also have an effect though this drops as the frequency is raised and disappears above $\sim 10^3$ Hz. The effect is anisotropic and (100) and (010) reflections are not influenced by a field, whatever its direction. Curiously, X-ray diffraction is very little enhanced!

Systematic experiments were done on relaxation of the diffraction enhancement at various temperatures in the range 8 - 71 °C; relaxation of the whole integrated intensity and of the height of different parts of the diffraction peak were separately studied. A particularly intriguing series of experiments involved heating a crystal from ~100 K with a field switched on and monitoring the diffracted intensity continuously. The enhancement effect does not show up till the temperature passes 200 K, and above this same temperature an increasing ionic current flows through the crystal.

This paper is accompanied by a second, from the Academy's Institute of Physics (Gu Benyean, Xu Zhengyi, Zhao Shifu and Zhang Andong, *Scientia Sinica* **22** (9), 1010; 1079). Here a d.c. field applied to α-$LiIO_3$ crystals is found to enhance the diffraction of light. This fact had been previously reported, and the effect depends on field polarity, field strength and temperature in much the same way as does neutron diffraction. The present paper works out the mathematical implications of the hypothesis that the d.c. field drives mobile impurity ions along the *c* axis until they come to rest along a series of layer-like defects (stacking faults?) lying parallel to the six pyramidal planes of the form {101}. The hypothesis is confirmed both by comparing the anisotropic diffraction patterns recorded in the presence of a field with calculated patterns, and by micrographs of a slab cut in such an orientation as to show a single family of defects edge-on.

The defect-piling hypothesis can presumably explain the neutron diffraction anomaly, though it is not altogether clear why that effect is restricted to the (0002) reflections, in view of the fact that the defects are not perpendicular to the *c* axis.

The literature cited in both papers indicates that the two laboratories have been systematically studying α-$LiIO_3$, at least since 1972. The initial motive may have been, at least in part, interest in the applications of this crystal in non-linear optical devices (see for example Sailor *Phys. Stat. Sol.* (*a*) **4**, K173; 1971), but the near success in confirming the origin of the various anomalies deserves attention from the various groups elsewhere studying ionic conductors.

Several of these conductors in fact contain lithium, and intensive research on several such crystals has been done in recent years, especially by a team at the Max-Planck-Institut für Festkörperforschung in Stuttgart. Some species show uniaxial preferred ionic conductivity, like α-$LiIO_3$: thus ß-eucryptite, $LiAlSiO_4$, has a hexagonal quartz-like structure with lithium ions arranged in channels parallel to the *c* axis, and conductivity $\parallel c$ is about 10^3 times higher than $\perp c$ (v. Alpen, Schulz & Talat *Solid State Comm.* **23**, 911; 1977; *Electrochim. Acta* **22**, 805; 1977). It would be most interesting to test whether α-eucryptite responds anomalously to d.c. fields in a way similar to α-$LiIO_3$, another uniaxial conductor.

Another Li-bearing ionic conductor, Li_3N, which has a layer structure of Li_2N and pure Li layers, has been extensively grown and investigated in Stuttgart, and a detailed survey of its properties has been published (Rabenau *Festkörperprobleme* **18**, 77; 1978). X-ray structural studies of quite remarkable precision (Schulz & Schwarz *Acta Cryst.* **A34**, 999; 1978; Schulz & Thiemann *ibid,* in the press) have shown that at room temperature the Li_2N layers have about 1% of Li^+ vacancies whereas the interleaving pure Li^+ layers are fully occupied. At 400 °C, the vacancy concentration in the Li_2N layers goes up to 4%. On this basis, the considerably higher ionic conductivity in the plane of the layers compared with that at right angles can be interpreted. This crystal species, therefore, has two-dimensional rather than one-dimensional conductivity, and if Li_3N shows any behaviour akin to α-$LiIO_3$, it may well obey quite different crystallographic laws.

The Stuttgart experience suggests that the best way to understand anomalous behaviour in ionic conductors is to combine a number of quite distinct experimental techniques focused on the same crystal species, preferably including structural analysis at a very high level of accuracy. This seems also to be the strategy that the Chinese are following in their study of α-$LiIO_3$.

Yang's interesting experiments on frozen impurity migration at low temperatures put one in mind of the little-known technique called Ionic Thermo Currents (ITC). This method, formally

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analogous to thermoluminescence analysis, involves cooling an ionic crystal in a field, switching off the field and then reheating the crystal connected to an ultra-sensitive electrometer which detects the microcurrent resulting from the randomisation of the aligned vacancy-impurity electric dipoles (Bucci & Fieschi *Phys. Rev. Lett.* **12**, 16; 1964; Strutt, Weightman & Lilley *J. Phys. E. Sci. Instr.* **9**, 683; 1976). This technique could perhaps be applied with advantage to α-$LiIO_3$, to monitor the microcurrents resulting from the randomisation of segregated ionic impurities.

It would be fascinating to know why X-ray diffraction intensities from α-$LiIO_3$ are affected by electric fields so much less strongly than are neutron diffraction intensities. Perhaps the active impurities will need to be identified and their atomic scattering factors for X rays and neutrons identified, before sense can be made of this anomaly within an anomaly. □

Standards for microwave radiation

from R. P. Blackwell

THERE has been growing concern in recent years over the possible biological consequences of exposure to microwave and radio frequency radiation, partly stimulated by the thousand-fold difference between the maximum permissible exposure standards current in Western and Eastern Europe. The much stricter Eastern standards have raised questions of safety in the West, but a realistic appraisal of the basis for this difference is hard to achieve.

Microwaves and radio frequency radiation are absorbed to a greater or lesser extent by biological materials, the energy appearing as heat; the predominant mechanism being dipole relaxation of water molecules. The photon energies of this radiation lie in the range 10^{-3} to 10^{-7} eV; orders of magnitude below the activation energies of molecular interactions (Cleary, *Health Phys.* **25**, 387; 1973). The generally accepted view is that the biological changes which are observed, such as cataract formation in the eye, are a result of heat production, although the view that non-thermal effects are important at low levels of exposure is held by some, particularly in Eastern Europe.

The Western standard of 10 mW cm^{-2}, which is considered to be the maximum safe exposure, was derived from thermal considerations. Because the body effectively deals with the transport of quantities of heat greatly in excess of the basal metabolic rate of 1 W kg^{-1}, whole body exposure at a power density which results in the dissipation of about 1 W kg^{-1} of heat is considered to be safe. The Soviet exposure standard seems to be the result of a different philosophy; their industrial safety standards often represent an ideal rather than a practical limit. They assume that any detectable biological change constitutes a hazard and consequently their exposure standard of 10 μW cm^{-2} was reached by adding safety factors to a power density at which no change has been observed.

Many Soviet papers report biological effects at power densities which do not seem capable of producing any thermal change, and are therefore often ascribed to 'athermal' effects. An appraisal of much of this research is difficult. Many reports which are quoted in reviews are not available in translation; most of those which have been translated are difficult to follow. Of the reviews available, that by Baranski and Czerski (*Biological Effects of Microwaves*, Dowden, Hutchinson & Ross, 1973) is among the most recent and comprehensive. Many of the reports of experiments performed by Soviet workers have been criticised, particularly in terms of dosimetry and experimental detail. For example many of the apparent effects on the nervous system detected by electroencephalography (summarised by Baranski and Czerski) need careful interpretation. The presence of recording electrodes during irradiation and simultaneous recording makes it impossible to rule out artefacts due to thermal and electrical stimulation by the electrodes themselves.

A large proportion of reports from Eastern Europe concern behavioural, electrophysiological and strongly subjective changes of a generalised nature, which are notoriously difficult to quantify. Fatigue, memory loss and loss of concentration have been reported. Such reports include, for example, surveys of the health of people exposed to low levels of microwave radiation in the course of their work. The body of evidence produced in the West is not in accord with many of those findings.

A recent publication of the National Research Council of Canada (*The Biological Effects of Radiofrequency and Microwave Radiation*, Assenheim *et al.*, NRCC 16448; 1979) provides an objective, well referenced synthesis of the important topics in this field. What is particularly useful is the attention paid to explaining the relevant backgrounds in physics and biology, and the philosophy behind the setting of exposure standards. Research in this field is complicated by the experimental problems of exposure and dose measurement. The interaction of electromagnetic radiation and biological tissues depends on several variables, including the wavelength of the radiation and the size and electrical properties of the tissues. Diffraction and reflection may occur at tissue interfaces, which may lead to focusing and resonance, with the consequent uneven distribution of heat, and formation of 'hot spots'. Assenheim *et al.* provide a comprehensive survey of the important topics in this area, dealing with both the prediction of absorption patterns and the techniques for temperature measurement required.

There is now sufficient material available, for example the *R. F. Dosimetry Handbook* of Durney *et al.* (Report SAM-TR-78-22, USAF School of Aerospace Medicine, Texas), to enable reasonable estimates to be made of the power distribution in a given situation. Thus it is now possible to extrapolate experimental data on biological effects to man, and to predict with a reasonable degree of accuracy the pattern of power deposition in man in most exposure situations, and the resultant biological effects, if any. Such information is necessary for the design and implementation of realistic, practical safety standards.

Many questions remain unanswered Perhaps one of the most interesting is posed by the work of Bawin *et al.* (*Ann. N. Y. Acad. Sci.* **247**, 74; 1975). They report that changes in the calcium ion efflux of chick and cat brain tissue *in vitro* can be modified by exposure to amplitude modulated VHF fields of 1 mW cm^{-2}. Unmodulated fields do not produce an effect; however electrostatic fields alternating at the modulation frequency (between about 6 and 30 Hz) do. These exposure values are somewhat lower than might be expected. This could have implications for effects on the central nervous system, if a similar process occurs *in vivo*.

There remain many problems associated with experimental work, particularly in terms of physiological measurement in a microwave field. Such measurements rely on transducers which normally contain some metallic components, and these may distort the field and lead to unpredictable power deposition patterns. It is possible that some of the confusion over low level effects is a result of this. For example, a metal coaxial recording electrode, implanted in cat cortex, can cause a complete shift in the pattern of power absorption, and an increase of two orders of magnitude in power deposition near the electrode tip.

Although the controversy over thermal versus athermal effects has not been completely resolved, it has had the effect of stimulating a re-appraisal of exposure standards throughout the Western world. One major development which any revised standard must take into account is the resonance effects which occur in man in the low VHF region. It is likely that more attention will also be paid to considering the amount of power that temperature-sensitive organs, or those with a poor blood supply — for example the lens of the eye and the testis — can safely dissipate. □

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Do nuclei have a neutron skin?

from John Edgington

EXACTLY 50 years ago the systematics of alpha-decay led Rutherford to the conclusion that the radius of the uranium nucleus must be less than 10 fm (10^{-14} m). An explanation for this incredibly small value, and an understanding of how and why it varied between nuclides, was a prime objective of the liquid-drop model. This viewed the nucleus as a close packed, incompressible array of neutrons and protons; the observed constancy of the binding energy per nucleon was seen as a consequence of the short range of the nuclear force and the resultant bonding between nearest neighbours only. The effect of this 'saturation' of bonds is that the nuclear radius increases as the cube root of the nucleon number, in agreement with earlier deductions from alpha-particle scattering. The more recent, and very extensive, measurements of electron scattering distributions have added flesh to this skeleton, and the distribution of electric charge within nuclei is now known quite accurately.

The distribution so revealed is remarkably model-independent. It is characterised by an almost constant central density, a rapid decline within one or two fm to a low value and an exponential decay thereafter. These features can be reproduced by any of a wide variety of shell models which, with their assumption of quasi-independent nucleon motion within some effective potential, have replaced the liquid drop model in structure calculations. The charge distributions themselves are thus too blunt instruments for the task of discriminating between the various models.

What then of the matter distribution, that is, the joint density of protons and neutrons? There is evidence, at least to first order in the electromagnetic corrections, that the strong forces between nucleons are charge independent, that is, indifferent to whether the particle is a proton or a neutron. One therefore expects protons and neutrons to have similar, if not identical, density distributions in nuclei. In terms of the single parameter which often summarises experimental results, their rms radii, $\langle r_p^2\rangle^{\frac{1}{2}}$ and $\langle r_n^2\rangle^{\frac{1}{2}}$ would be equal. Indeed, in a study of isobaric analogue states, Nolen and Schiffer showed 10 years ago that this equality holds quite well for a variety of medium to heavy nuclei (*A. Rev. Nuclear Sci.* **19**, 471; 1969).

Many subtle effects, however, conspire to produce this near-equality. For example, the destabilising effect of Coulomb repulsion causes all but a few light nuclei to contain fewer protons than neutrons; despite this, the excess neutrons do not contribute very much to the total strength of the pairwise n–n bonds. This follows from Pauli's exclusion principle which limits the interactions between identical particles. The total p–n bonding strength is not so limited. The presence of excess neutrons thus has little effect on the effective potential in which the neutrons move, although strengthening that experienced by the protons. Thus the protons are more tightly bound, and confined to a smaller radius. Conversely the Coulomb repulsion acting on the outer protons will tend to expel their wave functions and thus increase the protons' rms radius. The interplay of these competing effects, and others of lesser magnitude, is imperfectly understood, and in addition there is conflicting experimental evidence for a neutron-rich region, or 'skin', at large radii.

For example, isotope shifts in atomic spectra indicate only small differences, of varying sign, between the charge radii whereas similar shifts in the spectra of muonic atoms correspond to somewhat larger differences, the charge radius generally becoming greater as neutrons are added in heavier isotopes. The whole problem has been addressed afresh by the meson factories with their intense beams of muons and pions. For example, isotope shifts in pionic atoms have been measured at LAMPF, Los Alamos, and a sensitivity to radii changes of 0.02 fm is claimed. Unfortunately, in many of these experiments, the extraction of radii differences from the raw data is an opaque and model-dependent process, and theoretical uncertainties swamp the experimental ones.

A new method of measuring neutron radii has been reported from TRIUMF, Vancouver, which, it is claimed, is not only very sensitive to different values of $\langle r_n^2\rangle^{\frac{1}{2}}$ but is also virtually model-independent. In contrast to other radius determinations, this measures the neutron radius directly, not a difference between the charge and matter radii. R.R. Johnson *et al.* (*Phys. Rev. Lett.* **43**, 844; 1979) have scattered low energy π^- beams from pairs of isotopes, and measured the ratio of their differential cross sections. This ratio, it turns out, is sensitive to differences between the neutron radii of the two isotopes, almost regardless of what proton radius is assumed. The basic cause of this sensitivity is that the amplitude for π^-–n scattering is much larger than that for π^-–p at low energies, but the measurements at TRIUMF have, as a bonus, an additional enhancement arising as follows. The difference between π–n and π–p scattering arises from an interference between s and p-wave π–nucleon amplitudes, which has its maximum effect at extreme forward and backward scattering angles. Now in π-nucleus scattering a Coulomb-nuclear interference term is of course present, and Johnson *et al.* had already shown (*Phys. Lett.* **75B**, 560; 1978) that at very forward angles this interference is constructive for π^--scattering, but destructive for π^+. Thus any variations in neutron density distributions should show up with enhanced sensitivity in π^--scattering compared with π^+. Indeed a recent measurement of π^+–^{6,7}Li scattering at 50 MeV (*Phys. Rev.* **C18**, 2316; 1978) saw no such effects.

Johnson *et al.* scattered π^- beams of 30 and 50 MeV from ^{12,13}C and ^{16,18}O, and their results indicate that the heavier isotope has the greater neutron radius, by 0.04 fm and 0.21 fm in the two cases. Quoted errors of ±0.03 fm in each case include, for once, an estimate of the sensitivity to variations between the different models used in the calculations. The relatively small change between carbon isotopes is of particular interest.

Some other techniques for measuring neutron radii, such as studying the absorption of K^- mesons, find a gross neutron excess in the nuclear surface. In comparing the various methods one must bear in mind that the radial distances being sampled are very different; kaonic and antiprotonic atoms are probes of the farthest reaches of the nuclear surface, whereas the experiment of Johnson *et al.* is investigating the region of rapidly rising density close to the rms radius. It is to just this region that the various nuclear models principally address themselves, and this new evidence, the first measurement of $\langle r_n^2\rangle^{\frac{1}{2}}$ independently of $\langle r_p^2\rangle^{\frac{1}{2}}$, has an importance that is more than circumstantial. □

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Next in succession

from Peter D. Moore

MUCH current research on the process of succession in forests concentrates on the probability of a given species being replaced by an alternative species within a number of generations. This can lead to the development of a model describing a replacement series (see for example, Horn in *Theoretical Ecology* (ed. R.M. May) 187 Blackwell, Oxford, 1976). Certain non-linearities are quite frequently encountered, such as the persistence of populations from early in the sequence as a result of regular destruction of the forest, either by natural or human agencies. There are occasions on record, however, where such persistence of what one would normally consider an early successional tree species, can occur even in the absence of regular forest disturbance. The tree may even form the dominant component of an

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apparently stable community.

Perhaps the most familiar example in Europe is the birch forest of the north-western extremities of the continent, and which is best developed in northern Norway and Finland. This forest (described floristically by Hamet-Ahti, (*Ann. Bot. Soc. Vanamo* **34** (4); 1963) is an open scrub of birch trees 2–12 m in height, with no other tree species present, except scattered pine (*Pinus sylvestris*) in the more southern regions. Taxonomically there is some degree of confusion since at least two subspecies of *Betula pubescens* (ssp. *pubescens* and ssp. *tortuosa, sensu Flora Europaea* (ed. T.G. Tutin *et al.*) Cambridge, 1974) are present, but these are trees which would be replaced by others at lower latitudes.

It is of interest to note that arctic and subarctic forests dominated by birch also occur in certain subalpine situations, for example in the Carpathians, and in parts of the Eurasian steppes, but that they tend to be mainly associated with oceanic areas, such as Kamchatka in Pacific north-east Asia. In north America, the birches are accompanied by coniferous trees (such as *Abies, Picea* and *Larix*) and form pure stands only under disturbed conditions. The birch forests of northern Fennoscandia, by contrast, seem to have been stable over a considerable period. Fossil pollen data from these areas show an invasion of pine into the region between 8000 and 6000 years ago (Birks & Saarnisto *Boreas* **4**, 77; 1975) but the pine pollen levels attained by 4000 years ago are no higher than those of the present day (Hicks *New Phytol.* **78**, 715; 1977; Prentice *Boreas* **7**, 131; 1977). It seems therefore that the northern birchwoods have not developed in response to any human disturbance. The same applies to the birch woods of the northern part of the Isle of Skye in Scotland (Vasari & Vasari *Acta — Bot. Fenn.* **80**, 1; 1968).

As noted by Walter (*Vegetation of the Earth*, Springer-Verlag, New York, 1973) the coniferous trees of lower latitudes may be held back by a failure in seed set or by desiccation as a consequence of frost and wind. Such species as *Pinus sylvestris* extend into the birch forest zone along the sheltered valleys. Regeneration of birch, which is a light-demanding tree, probably depends on the death of adults, thus maintaining an open, light canopy forest.

Birch forests of this kind are not found in the southern hemisphere, although deciduous *Nothofagus* species which form woods in southern Chile may be considered an ecological equivalent (Godley *Proc. R. Soc. Lond* **B152**, 457; 1960). Recently an analogous vegetation type has been described from the Galapagos by Hamman (*Bot. J. Linn. Soc.* **78**, 67; 1979). Here an endemic woody composite, *Scalesia pedunculata* dominates the humid forests of Santa Cruz Island. The growth and population dynamics of this species have been investigated by means of permanent quadrats established in 1970 and recorded on three occasions since then. By this means the survival and growth characteristics of individual trees have been measured.

Mortality among *Scalesia* seedlings is high in the early years (only 40% survival after the first 2 years) and then decreases (2% survival after 7 years). Initial growth rates are very high, the trees reaching 7–8 m in 3–6 years. Height stabilises at about 10–12 m and girth increase becomes pronounced after about 5 years, coinciding with the onset of flowering. Few individuals survive beyond 10 years.

Many of these features such as short life span, rapid early height increase, and early flowering are characteristics normally associated with an early successional forest species. *Scalesia* persists and dominates the vegetation by means of a high seed production and a rapid population turnover. Although there is some disturbance of the forest by grazing animals, particularly giant tortoises, the *Scalesia* population does not seem to depend on this for survival. Vegetation zones on the Galapagos generally seem to be climatically determined (Hamman *Biotropica* **11**, 101; 1979) and the post-glacial climatic history of the Galapagos has been essentially stable as reflected by the vegetational history (Colinvaux & Schofield *J. Ecol.* **64**, 989; 1976). The *Scalesia* woodland should therefore be regarded as climax, in the sense that no further succession should be expected.

In both of the cases quoted, the Fennoscandian birchwoods and the Galapagos *Scalesia* forest, an over-riding biogeographical factor has restricted the invasion or success of those tree species which could be defined as late successional (or 'persistent multilayer' in the sense of Horn, *The Adaptive Geometry of Trees*, Princeton University Press; 1971). In the former case the restrictive factor is climate and in the latter it is geographical isolation. These simplified exceptions to the normal course of successional processes would repay more detailed investigation regarding regeneration and survivorship.

Inside the Earth

from David T. Rickard

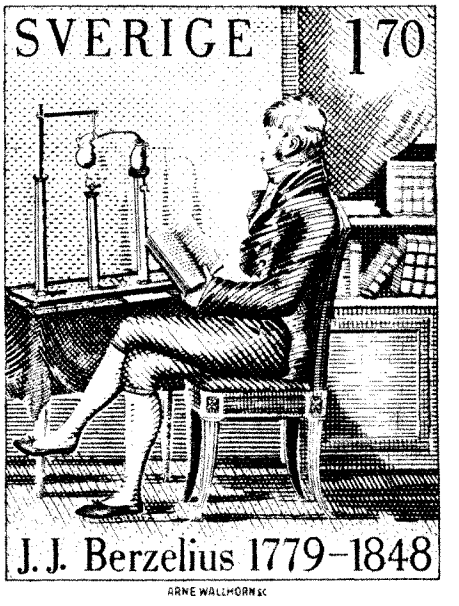
JÖNS Jacob Berzelius was born 200 years ago in the village of Väversunda in Sweden, and was to become one of Sweden's greatest, but least appreciated, scientists. Linnaeus, who had died the year before, represented the culmination of descriptive science; Berzelius' birth ushered in the analytical age.

One of Berzelius' major scientific contributions was his chemical system of mineralogy. Previously, minerals had been classified on the basis of form and colour in the same way as Linnaeus had classified plants. Berzelius chemically analysed minerals and classified them according to their compositions.

This introduction of chemistry into geological science was remembered by the Swedish Royal Academy of Sciences in their choice of a theme for a Nobel Symposium to commemorate the Bicentenary of Berzelius' birth. The Academy invited twenty-five distinguished scientists to discuss the chemistry and geochemistry of solutions at high temperatures and pressures.*

This subject is very much in the spirit of Berzelius. It represents an area of endeavour which only the most careful theoretical and experimental chemistry can hope to penetrate. It also reflects the conditions under which most of our planet exists.

The zone on the Earth where temperatures around 25 °C and pressures of about 1 atmosphere, or bar, predominate is



represented by a thin surface sheath about 12 km thick. Because we happen to inhabit this sheath our knowledge of inorganic and physical chemistry under these conditions is relatively good. However, the remaining 6,371 km down to the centre of the Earth are characterised by considerably higher pressures and temperatures. The PT (pressure-temperature) conditions in the first 250 km are illustrated in Fig. 1. It is in this high PT zone that the bulk of the Earth's rocks and ores are formed and where the major material transfer processes occur.

In contrast to 25 °C and 1 bar, our knowledge of inorganic and physical chemistry in the rest of the PT spectrum is

*The 48th Nobel Symposium was held at Bofors, Karlskoga, Sweden on 17–21 September, 1979. The Symposium proceedings are expected to be published in 1980. The symposium was organised by David T. Rickard and F.E. Wickman of the Geological Institute, Stockholm.

extremely limited. The symposium was therefore not a summary of achievements in a well-established and well-researched field. Rather it was a delineation of our areas of ignorance and a discussion of various methods of tackling the problems.

Take the best known fluid, water, as an example. S.D. Hamann (CSIRO, Melbourne) noted that our present knowledge of the PVT (pressure-volume-temperature) behaviour of water is known quite accurately up to 100 °C and 1 kbar, less accurately to 1,000 °C and 10 kbar, and progressively more uncertainly above these limits. The highest temperature reached by conventional techniques is 1,200 °C (at 4 kbar) and the highest pressure is 34 kbar (at 175 °C). Shock-wave PVT data, which are less reliable, have been obtained up to 400 kbar and 3,100 °C but are very uncertain above 30 kbar. The actual nature of water under these extended conditions is not well known. E.U. Franck (Karlsruhe University) suggested that treating it as $H_3O.OH$ was a good approximation; that is, as hydronium hydroxide, very similar to fused sodium hydroxide.

The data are even more limited for systems which contain other components in addition to water. The best-known system, H_2O-NaCl, has only been extended this year to 400 °C, 4 kbar and 25 weight percent salt. The few other systems that have been studied are known over a much more limited range of conditions.

Apart from the mechanical problem of confining solutions at high pressures and temperatures within a limited space, the solutions of interest are often highly corrosive. H.L. Barnes (Pennsylvania State University) pointed out that a major problem concerning geochemical experimentation was the relatively long reaction times often needed to reach equilibrium. Materials have to withstand temperatures of several hundred degrees and pressures in the kilobar range for up to one month. It is not without reason that these experimental systems are called bombs.

Materials in themselves are not the only problems facing the experimentalists at high temperatures and pressures. Experimental apparatus must be constructed so that measurements can actually be made. That is, it is not only necessary to confine the reactants but also to have access to them. Particular interest was engendered by spectroscopic methods of measurements of the chemical properties of solutions up to 5 kbar and 500 °C by M. Buback (Karlsruhe University). These techniques involve constructing a bomb with a window of a transparent, chemically resistant material, such as sapphire. Spectra of the reactant solution may be examined. Infrared adsorption spectra are commonly observed, but Buback also described the advantages and potential of laser Raman spectroscopy for these types of systems.

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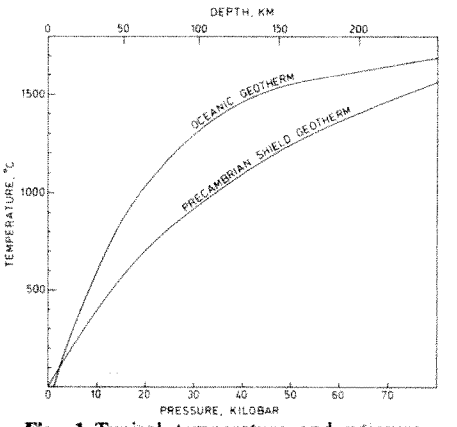


Fig. 1 Typical temperature and pressure variations with depth (geotherms) in the Earth for an oceanic and ancient Precambrian situation (from Wickman & Rickard, Chemistry and geochemistry of solutions at high temperature and pressure: an introduction to the symposium).

Even if experiments could be performed simply and quickly, the variety of solution compositions occurring in nature is so great that centuries of labour would be required to complete the work. Furthermore, it is not, at least at present, possible to examine experimentally the chemical properties of many systems under the extreme conditions that occur in the Earth. It is obvious that a major goal of high PT research is the construction of reliable methods for the theoretical prediction of the chemistry of solutions under extended conditions.

H.C. Helgeson and K.S. Pitzer, both from the University of California, Berkeley, presented alternative approaches to this problem. Helgeson discussed the 'ion association' approach, which provides specifically for ions combining together as a function of pressure and temperature. He argued that we know that solutions at high temperatures and low pressures contain a large proportion of combined or associated ions and that therefore the ion association model is more realistic.

The 'virial coefficient' approach used by Pitzer treats the solutions as though they contained only separate ions, and divergences from this ideal behaviour are accounted for in the coefficients of a single equation. Pitzer argued that the concentration of associated ions is not always unambiguously determinable and that there is essentially a continuous distribution of populations of ions at varying distances from one another.

However, these different approaches should not be regarded as competitive, but rather as complementary. For example, H. Eugster (Johns Hopkins University) showed, in a contribution to the discussion of Pitzer's paper, that use of the virial coefficient approach had solved for the first time a long-standing problem in physical chemistry — the theoretical prediction of salt precipitation during the evaporation of seawater. No other approach had resolved this problem which has been known since van't Hoff's work at the beginning of this century. In comparison, application of the ion association model has already given much information about the nature of geological processes under extreme physical conditions — information which is not available experimentally at present nor is likely to be for some time to come.

The application of theoretical and experimental high PT solution chemistry to geological and technological problems was a recurrent theme of the symposium. On the geological side delegates discussed the formation of igneous rocks, the origin of ore deposits and the response of rocks to solutions at high temperatures and pressures. Geothermal energy was a topic of some considerable interest and various aspects were reviewed from source to power station chemistry. T. Seward (DSIR, Petone, New Zealand) combined several themes in his poignant tale of drilling for hot water and finding a source that deposited gold in large quantities. His chemical studies were able to show how the gold was transported in these hot solutions, combined with sulphur-containing ions.

One of the prime purposes of the 48th Nobel Symposium was to initiate a dialogue between chemists and geologists working in the same field, often unaware of each other's contributions. In this the meeting succeeded and several firm suggestions were made for future cooperation and for future meetings on this theme. Berzelius would have been happy! □

Erratum

In the article 'Dissecting the red cell membrane skeleton' (*News and Views* **281**, 426; 1979) the sentence beginning on page 427, third column, four lines from the bottom, should read ". . This suggests that the lower *molecular weight forms are degradation* products of the parent band 2.1 molecule." On page 429, first column, the two lines following line 13 were inadvertently omitted. The complete sentence should read "This is probably the end of the molecule where actin and band 4.1 bind since interactions between acting and crude spectrin preparations are phosphorylation *sensitive (Pinder et al. Nature 270, 752; 1977). Unfortunately, so far at least, it has not been possible to recapture the phosphorylation* dependence in more purified systems (Brenner & Korn *op. cit.;* Cohen & Branton *op. cit.*). On page 429, line 9 of the second column is incorrect: the complete sentence should read "This implies that spectrin has evolutionarily critical functions and, since its only known functions relate to the *membrane skeleton, that the skeleton is an* evolutionarily critical structure".

Interferons and cellular regulation

from Mike Clemens

INTERFERON is best-known for its antiviral activity, but the principal message of a recent meeting on 'The Regulatory Effects of Interferon'* was that the multiplicity of other biological effects attributed to it are holding up now that pure interferons from several sources are available and have been tested.

Interferon of very high specific biological activity has been prepared after induction of its synthesis in either human fibroblasts (J. Heine & A. Billiau, University of Leuven; E. Knight, E.I. du Pont de Nemours & Co.) human lymphoblastoid cells (K. Zoon, National Institutes of Health, Bethesda or mouse Ehrlich ascites tumour cells (P. Lengyel, Yale University). Convincing evidence for homogeneity of the purified material was provided by amino acid sequencing studies conducted by M.W. Hunkapiller (California Institute of Technology). He described several technical improvements which have allowed identification of the first 20 N-terminal amino acids from less than 1 μg of interferons from each of the above sources. Interestingly, the human lymphoblastoid and fibroblast interferons show no sequence homology in this region (at either the protein or possible DNA codon level), whereas one species of mouse interferon does have some sequence similarities with human fibroblast interferon. Evidence was presented by D.C. Burke (University of Warwick) for the presence of the gene for human fibroblast interferon on the short arm of chromosome 9, rather than on chromosome 5 as has been previously suggested. As yet, however, no information is available concerning the structure of the interferon gene and reports on progress towards the possible cloning of the gene were conspicuous by their absence.

A great deal of information was presented on the effects of the various interferons on a wide range of cell types. More knowledge has been accumulated about type II or 'immune' interferon, produced principally by T lymphocytes in response to mitogenic or antigenic stimulation. Both S. Baron (University of Texas, Galveston) and E. Falcoff (Institut Curie, Paris) reported that it is antigenically distinct from virus-induced (type I) interferons. Many of its biological effects, including the induction of new enzyme activities in mouse L cells, are similar to those of type I interferon, but its ability to inhibit the growth of tumour cells is relatively higher.

The inhibitory effects of interferon on cell multiplication are also of particular interest. A.A. Creasey (Stanford University) suggested that, in some human melanoma cell lines, interferon may prevent a metabolic event required for commitment to a new round of DNA synthesis. The nature of this event and the mechanism by which it is modulated by interferon remain unknown. However, the series of 2′5′-linked adenylate oligonucleotides, synthesised by an interferon-induced enzyme in several cell types, has been implicated in this regulatory system. Inhibition of thymidine incorporation into DNA by the 2′5′ oligo(A) series was reported in L cells (A.G. Hovanessian, Institut Pasteur, Paris), concanavalin A -stimulated lymphocytes (A. Kimchi, Weizmann Institute, Israel) and human lymphoblastoid cells (I.M. Kerr, National Institute for Medical Research). Whether these effects are consequences of an earlier inhibition of protein synthesis remains unclear. Techniques for *in vitro* screening of the responsiveness of human ovarian carcinoma cells from patients for possible growth inhibitory effects of interferon were described by L.B. Epstein (University of California, San Francisco).

It is now clear that the interferons also have a wide range of effects on cells of the immune system. Those effects which may be particularly important for the anti-tumour actions of interferons include activation of natural killer cells (B.R. Bloom, Albert Einstein College, New York; G. Trinchieri, Wistar Institute, Philadelphia; R.B. Herberman, National Institutes of Health) and macrophages (S.I. Hamburg, New York University; M.A. Chirigos, National Cancer Institute). Tumour cells stimulate normal lymphocytes to synthesise interferon *in vitro*, and this has obvious implications for the control of tumour growth in the intact animal. A comforting conclusion which can be drawn from much of this recent work is that the various biological effects of earlier crude interferon preparations can also be elicited using the material purified to homogeneity (J. De Maeyer-Guignard, Institut Curie, Orsay; S. Pestka, Roche Institute, Nutley). Interferon has thus acquired a respectability which should stimulate further detailed molecular and cell biological analysis of its modes of action.

The recognition that interferon has a wide spectrum of hormone-like actions has also led to the realisation that it may exert harmful effects under certain circumstances. These may include some of the symptoms associated with certain autoimmune diseases, since a high proportion of patients with systemic lupus erythematosus or rheumatoid arthritis have elevated serum interferon levels (J.J. Hooks, National Institutes of Health). I. Gresser (IRSC, Villejuif) described the deleterious consequences of giving large amounts of interferon to new-born mice and summarised his view of these effects: "Interferon is a good thing. Too much of a good thing is sometimes a bad thing".

There have also been significant developments in understanding the anti-viral effects of interferon. There is now a considerable amount of information on the involvement of 2′5′ oligo(A) in the regulation of protein synthesis in interferon-treated cells. The interferon-induced enzyme responsible for synthesis of this series of oligonucleotides in the presence of double-stranded RNA (dsRNA) has been purified to homogeneity in the laboratory of P. Lengyel (Yale University). This group has also extensively purified both the endonuclease which is activated by 2′5′ oligo(A) and the protein kinase involved in inhibition of polypeptide chain initiation after interferon treatment. B.R.G. Williams and I.M. Kerr (National Institute for Medical Research, London) presented evidence that intact interferon-treated, EMC virus-infected cells synthesise 2′5′ oligo(A) and that the oligonucleotide will prevent virus replication when added to permeabilised infected cells. Thus the model which implicated 2′5′ oligo(A) in the antiviral actions of interferon, which was originally derived from studies with cell-free systems, has been confirmed in these whole cell experiments. L.A. Ball (University of Wisconsin), who has also extensively purified 2′5′ oligo(A) synthetase from interferon-treated chick cells, showed that the enzyme elongates the oligonucleotides in the 2′5′ direction non-processively, and will also add AMP residues to other small molecules such as NAD and ADP-ribose. C. Baglioni (SUNY, Albany, New York) described the structural requirements for dsRNA for activation of both the 2′5′ oligo(A) synthetase and the protein kinase. A minimum of 60 base-pairs of dsRNA is necessary and mismatching of bases lowers the efficiency of activation. It is not yet clear whether 2′5′ oligo(A) can inhibit viral protein synthesis selectively *in vivo*, for example by being synthesised and acting only in the vicinity of dsRNA-containing viral replication complexes, as suggested by Baglioni. In the case of Mengo virus-infected L cells host cell protein synthesis is also shut off, but later recovers in the interferon-treated cells (R. Falcoff, Institut Curie, Paris), perhaps because the 2′5′ oligo(A) is subsequently degraded by a cellular enzyme.

A more subtle way in which interferon inhibits the replication of tumour viruses was described by both P.M. Pitha (Johns Hopkins University, Baltimore) and R.M. Friedman (National Institutes of Health). Several steps in the maturation of these viruses are impaired in interferon-treated

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cells and the particles which are released show reduced infectivity. The latter phenomenon may be due to defective glycosylation of a virion protein. Interestingly, Friedman also reported that vesicular stomatitis virus produced by interferon-treated L cells is non-infectious, is deficient in G (glycoprotein) and M (matrix) protein content and appears abnormal in the electron microscope. □

Multiplying growth factors

from Denis Monard

MORE knowledge about the events regulating the initiation of DNA synthesis will certainly shed more light on the phenomena regulating the differentiation of animal cells which remains one of the main challenges of modern biology. Such basic knowledge should also contribute to understanding better how and why a tumour cell differs from a normal cell.

Opening a recent discussion* on this topic R.W. Holley (Salk Institute, San Diego) re-emphasised that all growth is regulated by a multiplicity of complex extra and intracellular events. The discovery of proteins which can trigger cell growth, such as migration factor, epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet growth factor has revealed that extracellular macromolecules have an important role as regulators of the cell cycle, and the number of such factors known is steadily increasing.

Platelets have provided several new growth factors. The stimulation of cell growth by three polypeptides released by platelets has been reported (D. Paul, Temple University, Philadelphia). All three polypeptides cross-react immunologically and chromatograph under the same conditions in heparin Sepharose. They differ, however, in their molecular weight. One of them, a basic protein, is displaced by β-thyroglobulin or EGF. But, in contrast to growth stimulation by EGF, its biological activity is impaired by heparin. Another growth factor has been obtained from platelet extracts (W.J. Pledger, University of North Carolina, Chapel Hill) but analogies with the other growth factors released by platelets are premature.

Virus-transformed BHK fibroblasts also produce and release two proteins which stimulate the migration and DNA synthesis of normal fibroblasts (P. Leuthard, Friedrich Miescher-Institut, Basel).

A cell line with an especially large number of EGF receptors has been used to purify a sarcoma growth factor (SGF). After being fixed with formalin, these cells still bind the growth factor released by cells transformed by mouse sarcoma virus. The growth promoting activity was then eluted from the fixed monolayer by a *p*H change. A homogeneous preparation of SGF, biochemically and immunologically distinct from EGF, could be obtained after only two elution cycles. Interestingly, SGF and EGF differ in their biological activity. In contrast to EGF, SGF stimulates the normal fibroblasts to assume a 'transformed' morphology and to form colonies in semi-solid medium, a characteristic attributed only to tumour cells (G.J. Todaro, NIH, Bethesda). This elegant modification of affinity chromatography with fixed cells should certainly allow the discovery of other proteins able to trigger the initiation of DNA synthesis.

It is possible at this stage to distinguish two classes of cell growth promoting proteins, that is, those released by normal cells which could be considered as the normal growth regulators and those released by tumour cells or by virus-transformed cells. More detailed biochemical and immunological analyses are now required to bring some order into the classification of such macromolecular growth factors.

In addition, greater knowledge of their mode of action would allow a better classification. However, the following facts reported at this round table discussion illustrate the complexity of such studies.

For example, growth-stimulating molecules such as prostaglandin $F_{2\alpha}$ and FGF can act synergistically in 3T3 cells (V. Richmond, Friedrich Miescher-Institute, Basel).

Cells also release high and low molecular weight factors which stop the cell cycle and, therefore might be able to antagonise or alter the biological effects of the growth factors. For example, a macromolecule has been isolated which reportedly arrests cells in G_1, an effect also produced by ACTH, thyrotropin or interferon (Holley).

Culture conditions also indirectly influence the sensitivity of cells to growth factors. For example, the number of receptors for EGF drops with increasing cell density and the binding kinetics are also affected (Holley). Moreover, viral transformation of cells can lead to a loss of EGF receptors (Todaro). It is also important to remember that cell growth regulation *in vitro* can be influenced by physical parameters which are not exactly similar to those predominant *in vivo*. For instance, the type of collagen used to coat Petri dishes can modify cell sensitivity to EGF and cell survival (W.R. Kidwell, NIH, Bethesda).

However, the macromolecular factors are apparently not solely responsible for the regulation of the initiation of DNA synthesis. Low molecular weight substances such as the classical hormones and nutrients (prostaglandin $F_{2\alpha}$, glucose, inorganic salts and so on) are also able to potentiate the effects of macromolecules or to elicit growth alone (Richmond; Holley). The field is now entering a phase in which one might consider as a working hypothesis, the existence of metabolic steps which are simultaneously triggered by all these different substances to initiate cell division. Nevertheless, it remains possible that the regulation of cell growth is due to a delicate balance between many chains of events which are not all metabolically linked. □

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*A round table discussion organised by S. Iacobelli and L. Jimenez de Asua on 'Regulation of initiation of DNA synthesis and differentiation in cultured animal cells' was held in Rome on 8 October, 1979.

Tokamaks still to the fore

from R.J. Bickerton

AT the 9th European Physical Society Conference on Controlled Fusion and Plasma Physics* the tokamak continued to hold the centre of the stage. Tokamaks are toroidal discharges maintained to a satisfactory level of stability by the superposition of relatively strong toroidal fields. The system has many advantages — not least the simplicity and ease with which hot, well-confined plasmas can be obtained. It has however two serious disadvantages. Powerful additional heating is needed to get high temperatures and the ratio of plasma pressure to magnetic field pressure is usually very low. The heating requirement has been met in the past with outstanding success by injecting powerful beams of fast (~40 keV) neutral atoms. Record ion temperatures of the order 6 keV were achieved in this way last year with more than 2 MW of injected beam into the Princeton tokamak PLT. But the longer term prospects for this technique are dim because for reactor scale plasmas higher energy beams are needed, making it technically difficult, expensive and inefficient. Thus great attention was given at the meeting to the new results on radio-frequency heating. In one set of experiments a few hundred kilowatts of power at the frequency of ion cyclotron resonance were injected into the tokamaks TFR (Paris) and PLT. In both cases the ion temperature was increased by several hundred electron volts with an efficiency comparable to that of low energy neutral injection. In this frequency range (20-60 MHz) there is no technical problem in generating the power; the main difficulty is in coupling the power to the plasma. Heating of tokamak plasmas at other plasma resonance frequencies was also reported, the most notable being the use of

*Held in Oxford on 17-21 September, 1979.

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100-200 kW of power at the first harmonic of the electron cyclotron resonance (~85 GHz) to raise the electron temperature in the Soviet tokamak T-10. In this frequency range the generation of high powers is the major difficulty; coupling to the plasma is through relatively simple wave-guide launchers. A general feeling at Oxford was that plasma heating in tokamaks by the injection of electromagnetic waves is at last becoming competitive with neutral injection. With the rich possibilities arising from a variety of frequencies it is hoped that an efficient method can eventually be found to deposit megawatts of power into the plasma core.

A second potential disadvantage of the tokamak arises from the same strong toroidal field that gives it its stability and general robustness. The ratio of plasma pressure to magnetic field pressure (β) tends to be very low, typically less than 1%. To be near economic a reactor needs β averaged over the volume ($\bar{\beta}$) to be in excess of 5%. New results included β values of the order 2-3%, seen in the ISXB (Oak Ridge), T-11 (Moscow) and TOSCA (Culham) devices. These values are obtained in circular cross-section devices and there are theoretical grounds for hoping that the higher values needed will be obtainable in non-circular cross-section systems such as JET. It should be emphasised that all three of these high $\bar{\beta}$ experiments are in effect limited by the available input power. Thus we only know that the values of $\bar{\beta}$ at which instability leads to a decrease in confinement are greater than those seen so far. Apart from these advances in tokamak heating and $\bar{\beta}$ there were many other results reported — notably the operation of the large Soviet tokamak T-10 at low values of q(~1.6) without a decrease in the confinement properties.

First results were reported from the new ETA-BETA II reversed field pinch experiment at Padua. This is approximately a one-third scale version of ZETA with some additional control of the toroidal magnetic field. To the surprise of some, the main features of the so-called quiescent period in ZETA have now been reproduced in this smaller device. Fluctuations in dI/dt are markedly reduced following field reversal and the electron temperature rises during quiescence from ~40 eV to ~100 eV. It will now be possible to study this quiescence phase in detail with modern diagnostics and hence to assess the potential of the reversed field system.

Research on the mirror system of magnetic confinement is now concentrated on two versions. The first is one in which the initial field of the mirror is reversed by plasma currents to give a final configuration which is similar to a compact tokamak without toroidal field and which has closed field lines and is therefore not a mirror at all. This is being studied at the Lawrence Livermore Laboratory and so far all attempts to achieve field reversal by pumping up the plasma pressure have marginally failed. New experiments are planned in which a low temperature plasma with field reversal is first created by a plasma gun and then heated by intense neutral beams. The second version is the so-called tandem mirror in which a long straight solenoid has intensely heated minimum |B| mirrors at each end. This line is also being studied at Livermore as well as in Japan and Novosibirsk. The aim of the tandem mirror is to create an electrostatic potential at the ends of the solenoid in such a way as to confine the ions. A major difficulty with all these open-ended systems is the heat conduction along the field lines by the highly mobile electrons. First results from the Livermore tandem mirror TMX were presented at Oxford. These show encouraging evidence of electrostatic plugging and of effective electron heat confinement. Thus the electron temperature in an end mirror is ~160 eV while in the solenoid the ion temperature is low (<100 eV), the density ~10^{13} cm^{-3} and the end loss small.

In the field of inertial confinement attention is now focused on ablative compression of complex pellets using as driver systems either lasers, electron, light ion or heavy ion beams. In the USA 10 kJ, 20 TW lasers at 1 and 10 μm wavelengths are now operating at Livermore and Los Alamos respectively. With the big Livermore laser (SHIVA) the D-T fuel has been compressed to ~160 times liquid density. These ablatively compressed cores are relatively cold and the next step will be to increase the implosion velocities through the use of higher power lasers on to larger targets. An interesting technique reported at the meeting was the use of radiochemical methods to deduce the density of the compressing shell. Transmutations are produced by the neutrons from the core and the subsequent radioactive decay is measured.

The choice of the best system is still open with lasers clearly ahead for research purposes but with embarrassingly low efficiencies for an ultimate reactor. All the particle drivers are more efficient but there are greater difficulties in transporting and focusing them to a target. If the energy gain from a single implosion can be made large enough then the efficiency of a CO_2 laser (1-5%) may be tolerable. Such high gains (~100) are theoretically possible from complex targets, but there is then the problem of making them cheaply.

For the first time in this series of conferences there was a report on the fusion programme in the People's Republic of China. Many were surprised by the size, breadth and sophistication of this work which includes mirror machines, laser compression and several tokamaks. The programme is gathering momentum after the setbacks of the Cultural Revolution. The presentation at Oxford was limited to a catalogue of devices but we can expect a serious contribution to fusion physics from China in the near future. □

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Erratum

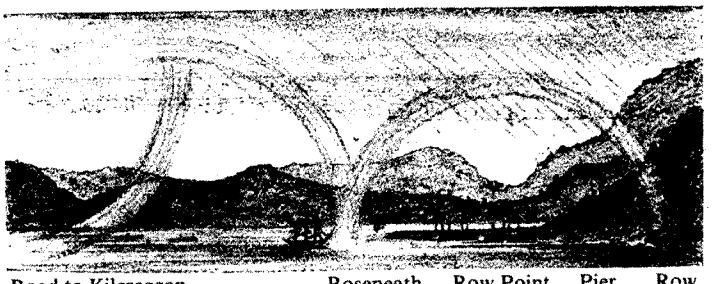
During printing a line was inadvertently ommitted from the article 'Road to unification' (*News & Views* **282**, 131; 1979). The first sentence in column 3, page 132 should read "Experimentally, weak neutral currents were discovered *at CERN in 1973*[19] *and parity-violation in neutral currents of precisely the right size was discovered* in the remarkable experiment[20] at SLAC last year." Our apologies to CERN.



100 years ago

A curious rainbow

I send you a rough sketch of a curious rainbow group seen in Gareloch about 8.25 a.m. on October 20. I would have written sooner but I delayed till I had obtained sketches from several different sources. I only saw the junction of the two bows at C, that being the only part of Row Bay visible from my standpoint, but several observers saw the whole group as I have drawn it. The sea was quite glassy, so that the inverted rainbow A must have been formed by the sun's rays reflected from the water. The wind was just beginning to rise and some scudding showers were passing up from the Firth of Clyde from the south-west, but the bay was quite calm.



Road to Kilcreggan. A Roseneath. B Row Point. C Pier. D Row.

The bow D was perfectly full and bright, while B died away at its highest point. I can only imagine that B was formed by light reflected by some bright cloud, but I did not observe any bright enough. The view is nearly north-west.

From *Nature* **21**, 20 Nov., 56; 1879.

review article

Brownian motions of flexible polymer chains

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The dynamics of flexible chains in solutions and in melts has recently been investigated using photon beat methods, inelastic scattering of neutrons, and also the stimulated Rayleigh effect. Relatively simple scaling laws have emerged, but some features of entangled systems remain unexplained.

THE stochastic motions of flexible polymer chains in a liquid phase are very complex. They fall into two classes: first, local motions, involving conformational changes inside a small number of adjacent monomers; second, large scale motions, where the whole polymer chain deforms and drifts in the neighbouring solvent.

The frequencies involved are in the range 10^{10} cycles for local motions, and 1–10^5 cycles for large scale motions. Here only the latter will be considered. These relatively slow modes have mostly been studied through mechanical experiments[1,2]. A very small concentration of flexible polymer is sufficient to increase significantly the static viscosity of a solvent; if we repeat this measurement at higher frequencies (using acoustic shear waves) we find an elastic component to the response with the chain behaving like an elastic spring. The frequency dependence of the response indicates a certain relaxation time of the chains. A similar time can be measured using certain mechano–optic and dielectric effects[3].

The 'photon beat' method has allowed the study of large scale motions. Here a very monochromatic laser beam (of frequency ω_0) is scattered inelastically from the polymer coils and the outgoing beam (frequency $\omega_0+\omega$) is probed at a well defined angle θ (or scattering wave vector $q=4\pi \sin\theta/\lambda$). The useful range of $\omega/2\pi$ is 1–10^6. The new feature of this method is the choice of a wave vector q. Qualitatively we may say that this method probes local fluctuations in the polymer concentration at spatial scales of order q^{-1} (ranging from 1,000 Å to a few microns).

Experiments using the weakly inelastic scattering of neutrons[4,5] have also become feasible; they give similar information, but for higher frequencies: from 10^{11} s^{-1} with 'back scattering spectrometers' down to 2×10^8 s^{-1} with the most recent 'spin echo' method. The range of q vectors available with neutrons is also different: for most experiments q^{-1} is between 10 and 100 Å. From these optical and neutron data, a physical picture has emerged for the stochastic motions of polymer chains, which is summarised below.

Dynamics of a single chain in solution

When considered at small magnification (low q) a polymer coil behaves likes a point-like brownian particle, with a certain diffusion coefficient D: this is conveniently measured by photon-beat experiments. For flexible coils in good solvents, D is found to be a decreasing function of the molecular weight M, and is often well described[6] by a relationship of the form $D\sim M^{-x}$ where x is of the order of 0.55. This does not agree with the classical theory for D (due to Kirkwood[7]) which predicts a Stokes–Einstein form:

$$D=\frac{kT}{6\pi\eta_0 R} \tag{1}$$

where T is the temperature, η_0 is the solvent viscosity, and R an effective radius of the coil. In a good solvent we would expect R to be controlled by excluded volume effects (giving a swollen coil): this gives $R\sim M^{3/5}$. The discrepancy in the exponent is beyond experimental errors, and remained a mystery for a long time until an attractive explanation was proposed by des Cloiseaux and Weill[8]. They note that in most of the usual solvents, which are not 'very good' the pair correlation function $g(r)$ between monomers of the chain, is of the ideal chain form $[g(r)\sim 1/r]$ for short distances $r<r_c$, while $g(r)$ decreases more rapidly at larger r: $g(r)\sim \text{const}\, r^{-4/3}$ $(R\gg r\gg r_c)$. This 4/3 law is the classic result due to Edwards[9], and reflects excluded volume effects typical of good solvents. The length r_c has been displayed in certain neutron diffraction experiments[10,11], and may be of the order of 20–100 Å (depending on the solvent quality). The main observation is that all static determinations of the coil radius are derived from an average of r_{ij}^2 between two arbitrary monomers

$$R_{\text{stat}}^2\sim \int r^2\, g(r)\, \mathrm{d}\mathbf{r} \Big/ \int g(r)\, \mathrm{d}\mathbf{r} \tag{2a}$$

while the dynamic properties are dominated by backflow properties[7]: the motion of monomer (i) induces a flow field near monomer (j) proportional to $1/r_{ij}$. This means that

$$\frac{1}{R_{\text{dyn}}}\sim \int \frac{1}{r}\, g(r)\, \mathrm{d}\mathbf{r} \Big/ \int g(r)\, \mathrm{d}\mathbf{r} \tag{2b}$$

The static average, equation (2a), is dominated by the contributions at large r, for which the excluded volume law behaves well. But the dynamic average, equation (2b), is more sensitive to the short range portion of $g(r)$, and for chains of not too large M, this makes R_{dyn} closer to the ideal chain value ($\sim M^{1/2}$). (Ultimately, at very high M, when $R\gg r_c$ the two values converge $R_{\text{dyn}}\sim R_{\text{stat}}\sim M^{3/5}$.) Future experiments using solvents of variable quality (variable r_c) should test the des Cloiseaux–Weill explanation in detail.

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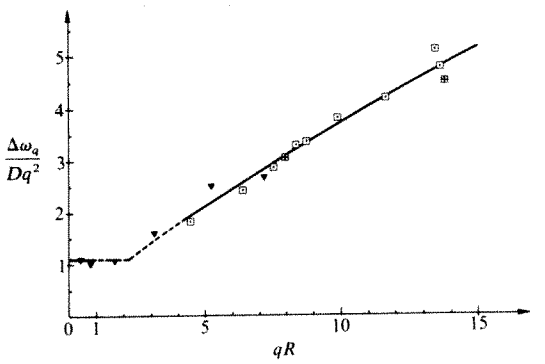


Fig. 1 Characteristic frequency $\Delta\omega_q$ for fluctuations of wavelength $2\pi/q$ in dilute polystyrene solutions. A scaling law is constructed by plotting $\Delta\omega_q/Dq^2$ versus qR where R is the coil radius and D the diffusion coefficient. Note that the points measured for two molecular weights (∇, $M = 8\times10^6$; $\square$, $M = 24\times10^6$) fall on one curve (after ref. 12).

The motions of the chain can be described in terms of an overall diffusion only when the probing length (q^{-1}) is much larger than the coil size R. The opposite limit has been studied[12] in very large polystyrene coils (with M values up to 2×10^7). The resulting plots of inelastic linewidth $\Delta\omega_q$ versus q (Fig. 1) can be summarised in the form:

$$\Delta\omega_q = Dq^2 f(qR) \tag{3}$$

where f is a dimensionless function. The long wavelength limit ($qR < 1$) is the diffusion law $\Delta\omega_q = Dq^2$ and imposes $f(0) = 1$. At the other end ($qR \gg 1$) we expect $\Delta\omega_q$ to become a characteristic frequency for a chain portion of spatial extension q, and to be independent of the overall chain size R. Because $D \sim 1/R$ from equation (1) then $f(x)$ must be linear in x for $x \gg 1$, giving:

$$\Delta\omega_q = \text{const}\,\frac{kTq^3}{\eta_0} \qquad (qR \gg 1) \tag{4}$$

This scaling law is followed relatively well by the data (although in practice the comparison is complicated by the des Cloiseaux–Weill correction). Theoretically, equation (4) was originally derived using a special model[13] for ideal chains, but it has a more general range of validity. Note the analogy between equation (4) and the rotational relaxation rate for a sphere of radius $q^{-1}[1/\tau_q = (kT/8\pi\eta_0)\,q^3]$.

Some measurements of $\Delta\omega_q$ have also been obtained from inelastic scattering of neutrons. As already mentioned, the results are limited to rather large $\Delta\omega_q$ values, and this imposes relatively high q: the data always fall in the domain $qR \gg 1$. Early work[14,15] was mainly concerned with melts and concentrated solutions, for which the situation is complex. Recent studies, using the 'spin echo' frequency analyser, have been performed on solutions of polydimethyl siloxane and polymethyl methacrylate, in benzene[16]. They give $\Delta\omega_q \sim q^z$ with z close to 3—in reasonable agreement with equation (4). Typical values of q are of the order of 0.1 Å^{-1}, hopefully small enough to warrant the continuum description (Fig. 2).

The above discussion on $\Delta\omega_q$ has been restricted to good solvents, where the chains were strongly swollen. In early theoretical discussions[2] the emphasis was more on the so-called 'Θ solvents' where the steric repulsions between monomers are exactly compensated by Van der Waals interactions: this led to simple random flight statistics for the coils ($R \sim M^{1/2}$) and eliminated the complications of excluded volume effects. However, another difficulty appears (at least for very long chains) in Θ conditions: as each coil is less swollen, it has a strong tendency to become knotted on itself. These knots could complicate the dynamics, the coil behaving as a whole like a small rubber ball[17]. Present experiments, however, have not shown any clear-cut effect of knots in Θ solvents, and this point remains unclear.

Diffusion in semi-dilute solutions

Let us now consider many coils in a good solvent, with a concentration c (g cm^{-3}) such that different coils overlap very strongly. The internal concentration of a single coil is of the order of $c^* = MR^{-3} \sim M^{-4/5}$ and the present regime corresponds to $c \gg c^*$. For large M values c^* is very small, and, the condition $c \gg c^*$ is achieved for a broad range of 'semi-dilute' solutions.

In these solutions, we can define two distinct diffusion processes: (1) 'cooperative diffusion' (D_{coop}) is associated with long wavelength fluctuations of the polymer concentration, and can be probed by photon beat experiments[12]. For instance, with polystyrene, in relatively good solvents, one finds a value of D_{coop} which is independent of molecular weight and increases with concentration: $D_{coop} \sim c^m$ with $m \sim 0.67$.

These are natural features: all the chains drift simultaneously, and behave as an elastic 'sponge' moving in a viscous medium. The size of the holes in the sponge (called the correlation length ξ (ref. 18) depends on c but not on M: both the elastic restoring forces and the friction forces involve only ξ and are independent of M in the semi-dilute regime: the same holds for D_{coop}. Furthermore, when c increases, the elastic rigidity of the 'sponge' increases very fast, and this explains the increase in D_{coop}.

It is easy to construct scaling laws for ξ and for D_{coop} (ref. 19). For the correlation length:

$$\xi = R\left(\frac{c^*}{c}\right)^n \qquad (c \gg c^*) \tag{5}$$

noting that for $c = c^*$ (close packed system of non-overlapping coils) the size of the interstices is comparable to the single coil radius R. Furthermore, we want ξ to be independent of M. Since $R \sim M^{3/5}$ and $c \sim M^{-4/5}$ this means that $n = 3/4$, a value confirmed by neutron diffraction data[18]. Similarly for the cooperative diffusion coefficient we can write:

$$D_{coop} = \frac{T}{6\pi\eta_0 R}\left(\frac{c}{c^*}\right)^{3/4} = \frac{T}{6\pi\eta_0\xi} \tag{6}$$

Equation (6) ensures that, for $c \sim c^*$, D_{coop} returns to the single coil value of equation (1) and the exponent is such that D_{coop} is independent of M. The remaining discrepancy between the theoretical exponent (3/4) and the experimental value for polystyrene ($m = 0.67$) is probably another effect of the des Cloiseaux–Weill correction.

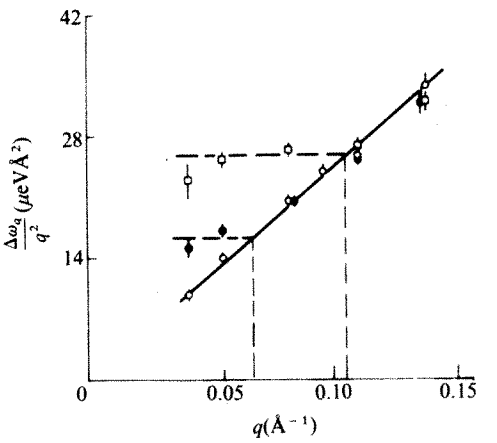


Fig. 2 Plot of $\Delta\omega_q/q^2$ versus q for three solutions of polydimethyl siloxane in benzene ○, PDMS/B 0.05 g; ●, PDMS/B 0.15 g; □, PDMS/B 0.3 g (after ref. 16). Note the difference in q values with Fig. 1: neutrons probe higher qs. The relevant curve of the present discussion corresponds to the most dilute solution (○). At higher concentrations and low q values ($q\xi < 1$) a more complicated regime appears.

The friction factor between the 'sponge' and the solvent can also be measured directly by sedimentation studies; data on polymer solutions have been collected for a long time[20] but it is only recently that a systematic study in semi-dilute solutions of low c has been carried out[21]. The sedimentation coefficient of polystyrene in good solvents is found to decrease like $c^{-0.59}$ while scaling would predict $c^{-0.5}$.

(2) 'Individual diffusion' (D^*) is associated with the brownian motion of one labelled chain immersed in a solution of chemically identical (but unlabelled) other chains. The diffusion coefficient D^* is rather small and very different from D_{coop}: it is a decreasing function of concentration and also of molecular weight. D^* is controlled by certain wiggling motions of the probing chain among the other chains, all being strongly entangled; therefore a theoretical study of D^* is difficult. I suggested previously[22] a simpler physical situation, where a single mobile chain moves in a fixed network of other chains: this has been called reptation. For the reptation model, D^* is proportional to M^{-2}. Various realisations of the model have been achieved[23]. However, the simplest idea was realised only recently: it replaces the network by a solution of 'ambient' chains, chemically identical to the probing chain, but longer: then the motions of the ambient chains are so slow that effectively they behave like a fixed network.

Two main experimental approaches have been used to study the very slow diffusion described by D^*. In the Klein experiments[23] the labelled chains were deuterated, and their concentration was measured by IR spectroscopy. The diffusion times involved were measured in months, and the diffusion lengths were ~ 2 mm. In recent experiments by Hervet, Léger and Rondelez[24], a photochrome was attached to the probing chain, and the diffusion was monitored through the stimulated Rayleigh effect.

The principle is as follows: at time 0, with a strong laser pulse, we create an interference fringe pattern (of wavelength $2\pi/q$) in the solution. The photochrome molecules are chemically modified in the bright regions, and this creates a periodic modulation of the refractive index; a phase grating is constructed in the fluid. We then cut out the power laser; the amplitude of the refractive index modulation Δn at a later time t decreases like $\exp(-D^*q^2t)$ where D^* is the diffusion coefficient. We monitor this amplitude with a probing (weak) laser, measuring the intensity of a Bragg reflection on the grating. This method detects diffusion over very small lengths ($2\pi/q \sim 1$ μm) and thus allows measurements of very small diffusion coefficients D^* (down to 10^{-9} cm^2 s^{-1} at present). The main difficulty in this programme is chemical: the attachment of a photochrome at the end of a polystyrene chain, prepared by anionic polymerisation is a delicate operation (performed under direction of P. Rempp). The labelled chains and the ambient chains were both polystyrenes of the same length. (This is not the ideal reptation situation, where the ambient chains would be longer than the probe. However, it has been argued that the difference is not essential[25,26].) It was found that $D \sim c^{-\alpha}$ where $\alpha = 1.7 \pm 0.1$.

It is possible to predict an exponent of this magnitude by combining the rules of reptation ($D^* \sim M^{-2}$) with a scaling ansatz:

$$D^* = \frac{T}{6\pi\eta_0 R}\left(\frac{c}{c^*}\right)^{7/4} \tag{7}$$

The form of equation (7) ensures that D^* crosses over correctly to the single coil D at $c = c^*$. The exponent 7/4 is then chosen to make $D^* \sim M^{-2}$.

In spite of its apparent success, equation (7) must still be considered with some suspicion: (1) the self diffusion D^* is associated with a natural relaxation time τ_r proportional to $R^2(c)/D^*$ (where $R(c)$ is the size of the chain at concentration c). Using known scaling laws for $(R(c)$ (ref. 2) this leads to $\tau_r \sim M^3$ (a typical reptation result[27,28]) (Fig. 3). However, most mechanical data on τ_r (ref. 1) suggest a higher exponent $\tau_r \sim M^{3.3}$. The discrepancy remains completely unexplained at present. (2) Similar scaling arguments could be applied to the viscosity η of the entangled solution: making η equal to the solvent viscosity η_0 when $c \lesssim c^*$, and demanding that η be simply proportional to τ_r (to M^3) in melts. One would then reach

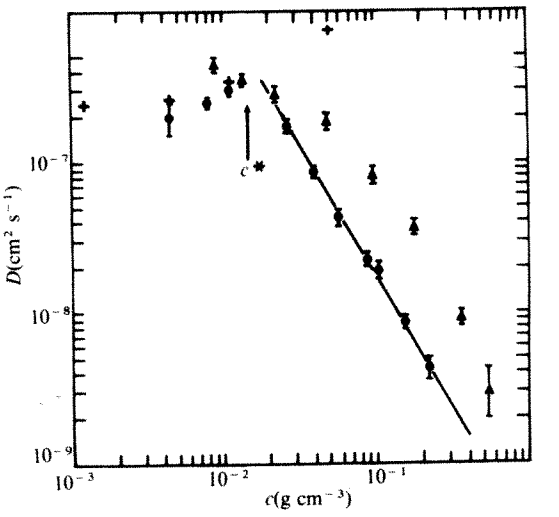


Fig. 3 Self diffusion coefficient D^* versus concentration in polystyrene–benzene solutions for two molecular weights (after ref. 24). ▲, $M = 123{,}000$; ●, $M = 245{,}000$; +, calculated from ref. 7; slope $\alpha = -1.75$.

$$\eta \cong \eta_0\left(\frac{c}{c^*}\right)^{15/4} \tag{8}$$

The actual exponent of $\eta(c)$ has not been measured in many clear-cut cases with low c^* values; but recent data (M. Adam and M. Delsanti, personal communication) suggest a higher exponent (~ 4.5). Thus either the scaling ansatz, equation (8), is wrong, or some minor complications occur: one of them may be due to a competition between monomer–solvent friction (described by η_0) and monomer–monomer friction: in equation (8) we might have to make the replacement

$$\eta_0 \rightarrow \eta_0 + \eta_1 c + \ldots \tag{9}$$

where η_1 describes monomer–monomer friction (and is independent of M); if the term $\eta_1 c$ dominates on η_0, even at relatively low c, the apparent exponent in equation (8) may be modified. However, if we accept this idea, we probably need a similar renormalisation of η_0 in equation (7) which might upset the agreement with the experiment.

Conclusion

The combination of the classical (mechanical) data on polymer dynamics, and other information (optics, neutrons) produces a reasonably consistent description of the long range, low frequency motions of polymer coils. However, (1) certain features, like the des Cloiseaux–Weill correction, or the viscosity renormalisation of equation (9), complicate the situation; (2) there remain some mysteries such as the possible role of knots in Θ conditions, or the exponent for the relaxation time $\tau_r(M)$ in entangled systems. The problem raised by $\tau_r(M)$ is central: as mentioned earlier, the experimental relaxation times are longer than expected. Polymers of high molecular weight may never be absolutely linear, but rather show some branching. Branched species cannot reptate freely, and are expected to have exponentially long relaxation times. One long branch is enough to achieve this; and a single branch point should be invisible by all traditional methods of detection (UV, IR spectroscopy, and so on).

Thus mechanical data on long, entangled chains may be dominated by a small number of uncontrolled branch points. However, this explanation is difficult to reconcile with the

apparent universality of the result: $\tau_r(M)$ is proportional to $M^{3.3}$ or $M^{3.4}$ for a wide variety of chemical structures.

Apart from branching effects, polydispersity may also be important: if the sample has a distribution of molecular weights M, the mechanical data in the entangled state are extremely sensitive to the high M tail of the distribution. We hope to see this point clarified by systematic studies on blends with a small fraction of very long chains in a matrix of smaller chains[26].

This review is largely based on a workshop on polymer dynamics held in Les Houches 1979 under the direction of J. P. Cohen Addad, and I thank the participants for many stimulating discussions.

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articles

Thermal minima along the axis of the Mid-Atlantic Ridge

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Mid-Atlantic Ridge basalts erupted near fracture zones seem to have lower quenching temperatures than along unfractured ridge segments probably because of enhanced cooling by exposing magmas to colder fault planes and more intense seawater circulation. The cooling effect is most pronounced along segments over hotspots with close ridge displacement fabric and recurrent rift jumping, probably reflecting additional complications, including subaxial magma flow about 'plumes', or the presence of an unusually large magma chamber on the Azores Platform and the southern part of the Iceland Plateau.

LARGE-SCALE heterogeneities in the abundance of heat-producing elements in the mantle should generate varying heat production rates. Given sufficient time after such heterogeneities have been produced, unusual melting conditions or convective flow patterns may develop in regions of the mantle richer in U, Th and K. Surface reflection of such unusual mantle activity may be the locus of the so-called 'hotspots' such as Iceland, the Azores and Galapagos regions which are characterised by more vigorous volcanic activity than adjacent mid-ocean ridges. The progressive increase in the content of K, U, Th of basalts collected along the Reykjanes Ridge and Iceland may reflect such mantle heterogeneity[1,2]. On average, Iceland basalts are enriched in K, U and Th by factors of 3.8, 6 and 9.6, respectively, compared with Mid-Atlantic Ridge basalts located south of 61° N. A significantly higher heat production rate is thus expected beneath Iceland relative to the Mid-Atlantic Ridge further south. However, it is uncertain whether such a higher heat production rate would manifest itself in generating higher heat flow, greater extent of melting, higher magma temperatures, unusual mantle convective flow, or a combination of these phenomena. It is also not known when these mantle heterogeneities were produced. High heat flow has been measured over Iceland[3] and steeper geothermal gradients have been inferred for Iceland compared with the Mid-Atlantic Ridge[4–6], but no direct heat flow measurements are yet available on the Mid-Atlantic Ridge crest. Lava production rates over Iceland are two to four times higher than on the Mid-Atlantic Ridge judging from crustal thickness and the Iceland topographic bulge[3,7–9]. This would indicate higher convective heat transport beneath Iceland than along the Mid-Atlantic Ridge. However, it is uncertain whether the higher basalt production rate on Iceland represents a higher degree of melting (melt–solid mass ratio) or segregation of interstitial melts from a larger volume of mantle rock beneath Iceland.

Lava temperatures are another useful way of investigating thermal phenomena, although also limited. Lava temperatures, whether directly measured or calculated from petrological data, only reflect temperatures of the magma at the time of eruption. The temperature of primary magma generated in the mantle is affected by cooling, partial crystallisation and possibly volatile loss as the magma ascends. The extent of polybaric fractional crystallisation of primary melts during ascent is a source of debate. While some workers maintain that all basalts have suffered extensive fractionation[10] others consider that some basalt compositions reflect relatively primary liquid[11]. Despite these limitations the determination of liquidus temperatures of basalts erupted along the Mid-Atlantic Ridge encompassing hotspots or fracture zones can be useful[12,13]. An estimation of the variation of temperature of magmas along the ridge may not only provide clues on thermal conditions in the underlying mantle, but also clarify the relative ascent rate and cooling

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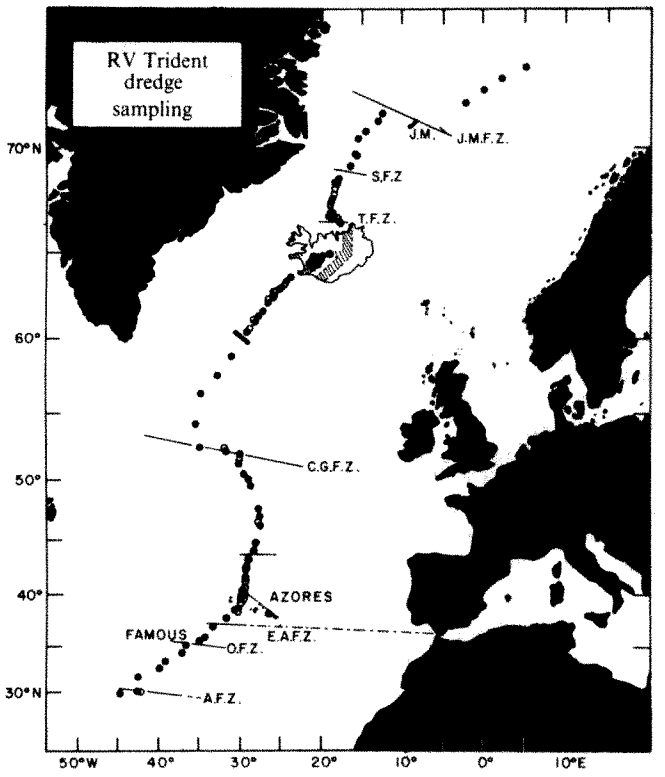


Fig. 1 Location of the Mid-Atlantic Ridge dredge stations used for the study (●). Not all locations contained glasses.

history of magmas in relation to tectonics. Further comparison of liquidus temperature variations along the ridge with other petrological and geochemical data, including heat producing elements U, Th and K, should also help to develop more realistic models for magma emplacement, ridge evolution and formation of unusual oceanic plateaus such as the Azores, Iceland and Jan Mayen. The first use of such an approach was made by Hermes and Schilling[12] along the Reykjanex Ridge and Iceland.

Here we examine the variation in calculated olivine crystallisation temperatures of 100 young Mid-Atlantic Ridge basalt glasses along the axis of the ridge from 29° N to 73° N, including traverses over Iceland (13 sub-glacial pillow lava localities from the western volcanic zone) and the Azores Plateau (Fig. 1). The submarine glassy rocks were dredged by RV Trident from 66 stations located either within the rift of the Mid-Atlantic Ridge or on the axis of the elevated crest of the Reykjanes and the Kolbeinsey Ridges. The stations are spaced at an average interval of 55 ± 63 km (1σ) and provide a reasonably representative sampling of recent magmatic activity on a 4,900-km long segment of the mid-ocean ridge system. Whenever possible, several samples were studied per station[14]. Liquidus temperatures were computed on the basis of the temperature-dependence of the equilibrium partitioning of Mg between olivine and co-existing glass[15–18]. The olivine crystallisation temperatures reported here are all based on the rim composition of olivine 'microphenocrysts' and surrounding glass in quenched pillow lava rinds and are considered to be realistic approximation of temperatures of Mid-Atlantic Ridge basalts at or close to the time of extrusion. Detailed discussion of this electron microprobe study, including the methods and the validity of the calculated temperatures of olivine crystallisation, are reported elsewhere[14].

Petrology

Phenocrysts are present in the majority of the glassy basalts studied. Modal analyses show that total phenocrysts are generally < 10 vol.% in the variolitic zone of the pillows, with maximum phenocryst content up to 23% near Iceland, the Azores Plateau and the Gibbs Fracture Zone (Fig. 3). Plagioclase phenocrysts are generally more abundant than olivine, and clinopyroxene is uncommon except over and near the Azores, Iceland and Jan Mayen plateaus. The composition of the olivine microphenocrysts studied ranges from 71.8 to 88 mol% fosterite, with a mode at 85 mol% Fo. A good linear relationship exists between the Fe/Mg content of these olivines and that of the glass. The corresponding K_D equal to 0.27 ± 0.01, 1σ suggests that equilibrium conditions prevailed[14]. (The Fe^{2+}/Fe^{3+} values of the glasses were estimated on the basis of an oxygen fugacity equivalent to that of the quartz + magnetite–fayalite exchange reaction at the liquidus temperature calculated from the Mg partitioning, using Waff and Weill's empirical relationship (unpublished data). The Fe^{2+}/Fe^{3+} obtained this way compared well with those actually measured on whole rock by a wet chemical method (Schilling *et al.* unpublished).

Within the normative classification scheme of Yoder and Tilley[19] the bulk compositions of the basalts studied correspond to either quartz–hypersthene or olivine–hypersthene normative basalts, and only two Ne-normative lavas were observed near 33–35° N. Stations TR119-8D recovered basalts which are just nepheline normative, and the glassy rims of picritic basalts at station TR123-4D are also just nepheline normative whereas the whole-rock is not. As expected, glass compositions are slightly more evolved than bulk rocks. Silica content of glasses ranges from 47.4 to 53.1% (mean 50.9 ± 1.0, 1σ) and in bulk rock analyses from 46.9 to 52.0% (mean 49.9 ± 1.0, 1σ). The chemistry of these pillow lavas, as well as of the glasses, reveals two major compositional groups which may be related to distinct petrological provinces (Fig. 2). One group forms a narrow iron enrichment trend with little change in alkali content for the region of the Mid-Atlantic north of the Gibbs Fracture Zone up to 70° N including Iceland (group *A*); the other group shows a notable enrichment and significant scatter in soda and comparatively more limited Fe enrichment and comprises basalts from the segments of the Mid-Atlantic Ridge between 53° N to 28° N including the Gibbs Fracture Zone and the Azores Plateau, as well as from 70° N to at least 73° N, which includes the Jan Mayen region and the Mohns Ridge (group *B*). Group *B* basalts can further be subdivided into ridge segments with distinct content of soda at equivalent Mg-value. The difference between the two groups is amplified in the more evolved magmas. The forsterite content of olivine from group *A* varies from 87 to 72 mol% Fo, and from 88 to 74 mol% Fo for group *B*. At equivalent olivine compositions, the Mg and Fe content of group

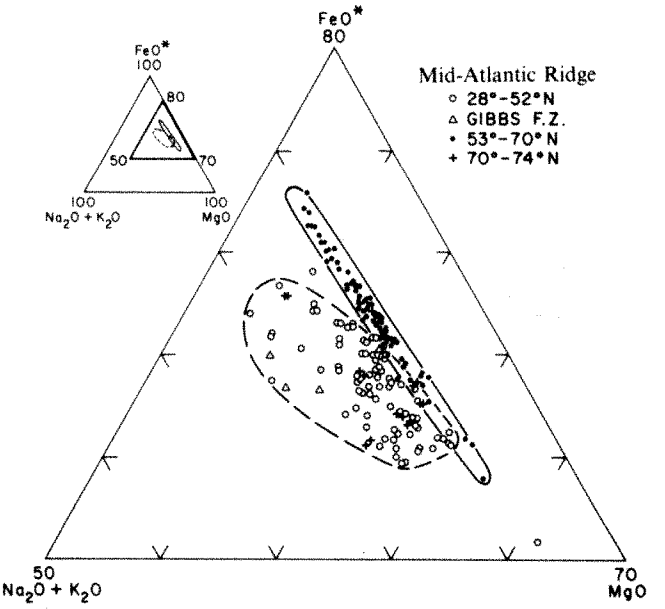


Fig. 2 Relative Mg, Fe, alkali content variation of the Mid-Atlantic Ridge basalt studies based on whole-rock analyses. Not all basalts are glassy.

A glasses is consistently higher than in glasses of group B, over the entire compositional range. Consequently, the olivine/glass partition coefficients for both Fe and Mg are higher in group A than in group B, but the corresponding K_D values for the two groups are indistinguishable within the error quoted earlier. The differences in olivine/glass partitioning of Fe and Mg between groups A and B can be attributed to melts composition, particularly differences in SiO_2 (or Si/O ratio) which is generally higher in group A basalts at equivalent indices of fractionation[14]. These observations also suggest that the Fe and Mg partition coefficients are affected to a similar extent by composition, since K_D is the ratio of these two partition coefficients.

Temperature variations along the Mid-Atlantic Ridge

We have calculated the crystallisation temperatures of olivine microphenocrysts in Mid-Atlantic Ridge basalt glasses by using the temperature-dependency of the olivine/melt (glass) partition coefficient (D) for Mg defined as D_{Mg} = weight fraction of Mg in olivine/weight fraction Mg in liquid. The temperature dependency of the Mg partition coefficient has been experimentally determined at one atmosphere under controlled fugacity of oxygen for a wide range of basaltic compositions[15–18]. The relationship is of the form: $\log D = A/T\ (K) + B$. These studies have also shown that the compositional dependancy of D_{Mg} is small. The pressure dependancy remains poorly studied. We have calculated the temperature of crystallisation of the olivine '*microphenocrysts*' using the one-atmosphere D_{Mg} calibration of Fisk *et al.*[18]. This calibration was obtained on the Reykjanes Ridge basalts, which should further minimise possible compositional dependency of D_{Mg} in the case of the Reykjanes Ridge glasses and other glasses from group A. There are several uncertainties in the calculated temperatures of olivine crystallisation: (1) uncertainties in parameters A and B for the linear regression correlation between D_{Mg} and temperature can be as large as 30 °C between various calibrations[15–18]; (2) uncertainties due to analytical precision for MgO may translate into ± 5 °C error on the calculated temperatures; (3) uncertainties due to the compositional variation of individual olivine microphenocrysts has been examined by compilation of average core and rim compositions for 144 Mid-Atlantic Ridge basalts as well as intensity line scans for Fe and MgKα across selected microphenocrysts. The number of olivine analyses per sample ranged from a minimum of 6 to >20. Zoning was taken to be significant when the difference in core and rim composition exceeded the precision of the microprobe analysis method (± 0.25 mol% Fo). On this basis 40% of the samples have olivine microphenocrysts showing significant zoning. Of these, 49 samples exhibit normal zoning (decreasing forsterite content from core to rim) amounting to average of 1.4 mol% Fo, which corresponds to an ± 5 °C error on the calculated temperature. Reverse zoning was detected in 10 basalts, with average range of 0.75 mol % Fo. Maximum normal zoning observed was 2.82 mol% Fo (or an absolute uncertainty of 10 °C on calculated temperature), and maximum reverse zoning of 1.50% (a 6 °C absolute uncertainty). The composition of the glasses was determined by a minimum of three probe analyses of the glass, well away from crystal phases. Only average compositions of olivine 'microphenocryst rims' were used to calculate the Mg partition coefficients.

Variations in temperature of crystallisation of olivine along the ridge using this method are shown in Fig. 3. Based on our analysis of errors and uncertainties and systematic analytical procedure, we estimate that individual temperatures shown in Fig. 3 should not deviate by more than ± 8 °C on a relative basis. The temperatures range from a maximum of 1,225 °C in an Icelandic picritic glass (IC-117) to a minimum of 1,067 °C in quartz-normative tholeiite glasses on the southern margin of the Azores Plateau at 38 °N (TR154-10D). These values are within the range of liquidus temperatures of basic igneous rocks determined in the laboratory[20]. Temperature variations on a single site were evaluated from data on glasses taken in 15 dredges from which two or three samples were selected for study. The temperature difference among several glasses from a single dredge varies from zero to 33 °C and two groups of sites can be recognised. Five of the dredge stations contain heterogeneous samples, with dissimilar glass compositions, petrography and crystallisation temperatures; with temperature differences between samples of 11–33 °C. These dredges have clearly sampled several unrelated volcanic units. Ten dredges, for which multiple temperature data are available, contain samples with temperature differences in the range 0–6 °C. Other criteria, such as glass chemistry and petrography, indicate that these dredge stations contain several samples of the same volcanic unit.

Basalts with highest olivine crystallisation temperatures at each site have been joined by a line to yield a temperature profile of the ridge (Fig. 3). Also shown in Fig. 3 are: a bathymetric profile of the ridge based on water-depths at the dredge stations; the Mg-value of the basalts; the volume % of phenocrysts based on modal analyses (Schilling *et al.* in preparation), and the La/Sm variation as an index of mantle source heterogeneity[1,21].

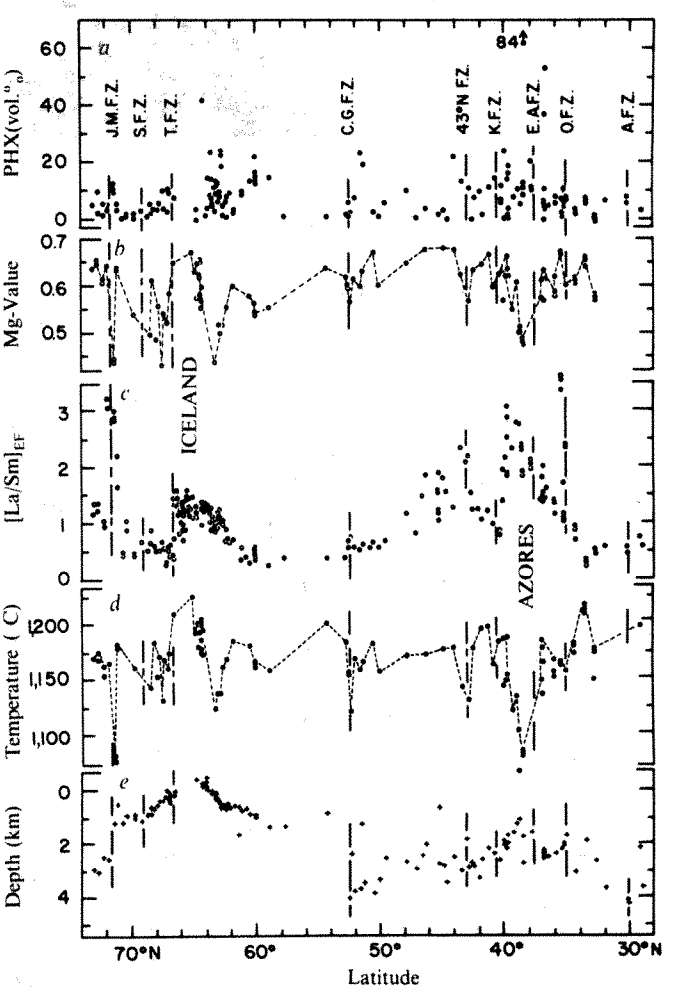


Fig. 3 Variation along the Mid-Atlantic Ridge, from top to bottom of: (*a*), total phenocryst volume per cent based on point counting modal analyses (Schilling *et al.* in preparation); *b*, $Mg/Mg+Fe^{2+}$ of glasses (atomic and using the method described in the text for estimating the Fe^{2+}/Fe^{3+} ratio)[14]; *c*, (La/Sm) enrichment factor relative to chondrites (refs 1 and 21 and Schilling *et al.* in preparation); *d*, calculated temperature of olivine and glass[14], using the calibration of Fisk *et al.*[18]; and *e*, water depth at the dredge stations. Note low temperatures at fracture zones, and the two well defined minima south of the Azores and Iceland plateau. Abbreviation for fracture zones (FZ) are: JM, Jan Mayen; S, Spar; T, Tjornes; CG, Charlie-Gibbs; K, Kurchatov; EA, East Azores; O, Oceanographer; and A, Atlantis.

The most notable feature of the temperature profile along the Mid-Atlantic Ridge is the tendancy of significantly lower temperatures at or near fracture zones. However, three possible minima are suggested by single dredges only; the Jan Mayen Fracture Zone (1,078 °C), the Gibbs Fracture Zone (1,122 °C), and possibly the 43° N fracture zone (1,131 °C). Although the existing sampling density along the ridge prevents us demonstrating statistically that lavas erupted at or near fracture zones are systematically lower, this trend is sufficient to warrant further discussion and testing. Furthermore, the lower olivine crystallisation temperatures observed for fracture zone basalts are in keeping with the occurrence of more evolved basalts in other Mid-Atlantic Ridge transform fault zones, most probably as a result of fractional crystallisation[22]. Some variation in the extent of partial melting along the ridge is also likely. Shallow depth fractional crystallisation may affect significantly the Mg value of magmas but has only a minor effect on the La/Sm ratio[21,23,25] (Fig. 3). On the other hand La/Sm is more and the Mg value less susceptible to variation in extent of partial melting[24,25]. Some local correlation may exist between La/Sm and the Mg value or the temperature of olivine crystallisation as a result of the combination of these two processes. However, the large amplitude and wavelength variations in La/Sm shown in Fig. 3 are primarily reflections of mantle source heterogeneities in this ratio beneath the ridge and clearly do not parallel the Mg value[21,25].

Two pronounced and statistically better defined thermal minima are also observed on the extension of the Reykjanes Ridge over the southern edge of the Iceland Plateau (~63°10′N) and the ridge transect over the southern part of the Azores Platform (~38°30′N) (Fig. 3). The temperature minimum south-west of Iceland first observed by Hermes and Schilling[12] was attributed to shallow-level fractional crystallisation. The presence of another well-defined minimum over the southern part of the Azores Plateau[13], again another hotspot, suggests the possibility of a common cause for such phenomena. Both minima share the following characteristics: (1) they occur on or over the flank of two major volcanic plateaus or topographic bulges; (2) they are probably underlain by a thicker crust than normally present along ridge segments further remote from the Azores or Iceland[3,5,26]; (3) the axis of these two ridge segments is displaced in en-echelon fashion by a series of small fracture zones[5,21]; (4) magnetic anomaly lineaments typifying ridge axes have lost coherency along these two ridge transects[5,27]; (5) complex recurrent rift jumping has also been suspected in these two regions[28]; (6) both ridge segments are geochemical transition zones characterised by large-scale radiogenic isotope and La/Sm gradients (Fig. 3) which have been interpreted as reflecting preferential flow and mixing beneath the ridge axis of mantle-plume derived with LILE-derived material[1,21]; (7) basalts erupted in these two regions are more evolved, have lower Mg values and contain larger proportions of phenocrysts than other MAR basalts, including particularly clinopyroxenes (Fig. 3; and Schilling *et al.* in preparation); and (8) basaltic glasses defining these two temperature minima also contain two populations of olivine and plagioclase phenocrysts. Macrophenocrysts have common resorption features suggestive of magma mixing[14], whereas late generation microphenocrysts do not.

Origin of thermal minima near the Azores and Iceland hotspots

Models describing the cooling of the crust–lithosphere along normal ridge segments, where volcanism occurs passively in response to plate divergence, have been treated primarily as a two-dimensional thermal problem in a plane perpendicular to a semi-infinite ridge axis[29–32]. We suggest that on or near 'hotspots' where subaxial and additional tectonic complication seem to occur[1,8,21], and across fracture zones, lithospheric cooling must be treated as a three-dimensional thermal problem. More efficient cooling in ridge segments cross cut by fracture zones is to be expected as the active ridge segment abuts against colder lithosphere across the transform fault. More extensive fracturing should also enhance circulation of seawater and heat exchange with magma chambers beneath such regions[33]. It has been suggested that the flux of 'mantle plume' material located beneath a ridge is largely independent of spreading rate of the plates above[34]. As a result, when the flux of mantle plume material per unit ridge length exceeds the rate of plate divergence by seafloor spreading above the plume, the excess material derived from the plume is accommodated in several possible ways: (1) by formation of an elevated ridge axis (rather than a rift valley) along the ridge branching from the plume; (2) stagnation and cooling of magma in unusually large magma chambers at the base of the crust; (3) development of a thicker crust above and about the plume[1,21]; and (4) lateral flow of magma derived from the plume, subaxial to the ridge, which mixes with complementary melts derived from the LILE-depleted asthenosphere[1,8,21,35,36]. Modelling and sorting of these different effects are difficult with our present knowledge. An attempt to evaluate the thermal effect of magma flowing and mixing beneath the ridge axis about these two hotspots is made in the following section.

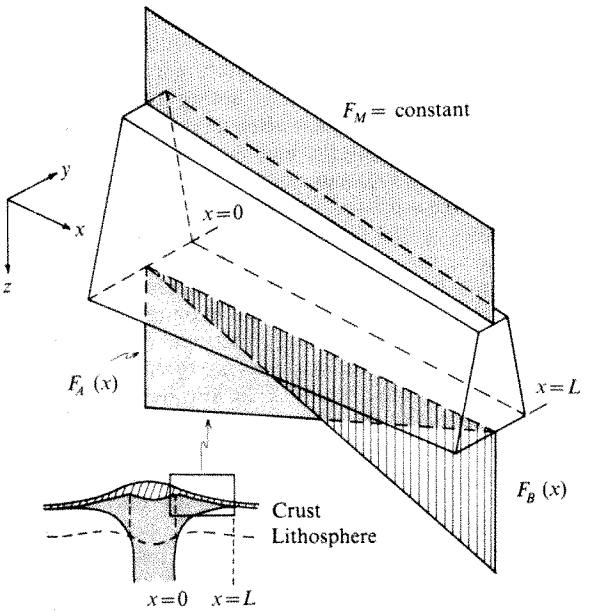


Fig. 4 Model for flow and mixing of magma in subaxial magma chamber. Flux variations with x of the two magma types entering the magmas chamber are shown as shaded areas: Grey shading indicates plume-derived magma type A; vertical strips indicate magma type B derived from the LILE-depleted asthenosphere; and dotted area show flux variation with x of mix-magma type M leaving the magma chamber and solidifying as new oceanic crust. Magma flow directions are not shown. Magma type A may enter partly from below or from the end face at $x = 0$ and flow along x. Magma type B probably enters mostly from below; whereas mix-magma M partly leaves the magma chamber from its top during dyke injection, or solidifies along both sides due to lateral crustal spreading in the y direction.

Several mechanisms have been suggested for flow of magma along rifted crustal zones. Vogt[35] has modelled sub-crustal and axial pipe-like flow down-slope about hotspot bulges on the basis of gravity and topographic gradients. Fiske and Jackson[37], Schilling[1] and Sleep and Rosendahl[38] have considered dyke propagation due to non-isostatically compensated topography which causes horizontal deviatoric tension. Direct evidence for intra-crustal flow of magma over 70–100 km distance within Iceland has been provided by Sigurdsson and Sparks[39]. Whatever the exact cause and nature of the transport mechanism, progressive cooling and crystallisation of the magma is to be expected during flow along the rifted zone. En echelon ridge axis displacements or recurrent rift jumping near hotspots will further complicate the nature of magma plumbing and enhance

cooling as discussed earlier. The subaxial transport process is also likely to be sporadic[8,40]. The material flowing may be entirely liquid or composed of a mixture of melt and solids (xenocrysts and/or phenocrysts). Cooling and partial crystallisation in the magma chamber, after mixing of the two magma types derived from the plume and the LILE-derived asthenosphere, is also likely to occur and obscure simple geochemical tests for the magma mixing model proposed. Clearly an exact solution of this complex thermal problem is not possible at present. Instead, we consider an empirical working model to account for the two thermal minima, which takes into consideration the preferential subaxial flow of plume-derived magma (or slush), its progressive cooling, fractional crystallisation and mixing with LILE-depleted magmas along the flow path.

Let the cartesian coordinate x be the horizontal direction along the ridge axis, z the depth and y the horizontal direction perpendicular to the ridge axis. Visualise an elongated magma chamber near the base of the crust beneath the ridge axis (Fig. 4). The magma chamber is fed in part by sub-horizontal flow of plume-derived magma A, whose flux F_A per unit length x decreases in intensity along the axis x, and in part fed from below by magma B from the LILE-depleted source, with a flux per unit length F_B increasing in intensity along the axis x. Mixing of the two types of magmas occurs within the magma chamber. The mixing proportions along x can be expressed in terms of the function

$$S(x) = F_A(x)/F_A(x) + F_B(x) \quad (1)$$

We set $x = 0$ at the periphery of the plume (chosen arbitrarily) and $x = L$ where the negative La/Sm and $^{206}Pb/^{204}Pb$ gradients[2] end some 400 km south-west of Iceland[1,2], or 1,000 km south-west of the Azores[21] (Figs 3 and 5). To satisfy the La/Sm and isotopic gradients along x, the function S must decrease from $S = 1$ at $x = 0$ to $S = 0$ at $x = L$. This presupposes that magma chambers over the plume are composed of melts derived entirely from the plume, whereas at $x > L$ magma is derived entirely from the LILE-depleted asthenosphere source[1,21]. The form of the function $S(x)$ between these two limits can be estimated by comparing the predicted La/Sm (or $^{206}Pb/^{204}Pb$) curve based on the binary mixing model, with the actual gradient observed using different empirical forms of $S(x)$ (Fig. 5). A linear function of the form

$$S = 1 - x/L \quad (2)$$

seems reasonable as a first order approximation. Finally we assume that the mixture of mass M per unit time and axial ridge length x (F_M) leaves the top of the magma chamber to generate new sea floor at constant rate along x. The system is assumed to be at a quasi-steady state, therefore

$$F_M = F_A(x) + F_B(x) \quad (3)$$

is constant and independent of x.

We assume that the net thermal result of the subaxial flow of plume-derived magma in the x–z plane is a decrease of the temperature $T_A(x)$ of this material from $T_{A,0}$ at $x = 0$ to $T_{A,L}$ at $x = L$. There are two reasons for making this assumption: First, the temperature of the country rock about a plume is likely to decrease outward[41]; second, the longer the path of travel of magma A outwards from the hotspot, the cooler it is likely to become. The temperature of LILE-depleted magma (T_B) is assumed to be constant along x, judging from the smaller range of liquidus temperature variations along normal ridge segments characterised by basalts with low La/Sm ratios (Fig. 3).

We further assume that: the two masses of magma type A and B have the same heat capacity (the heat capacity dependence on temperature is also ignored); and that the heat dissipated from the mixing of the two magma types is negligible. The net temperature of the magma mixture $T_M(x)$ after mixing of masses A and B is:

$$T_M(x) - S(x)T_A(x) + [1 - S(x)]T_B \quad (4)$$

The exact form of T_A with x is, of course, unknown. We can, however, set a minimum temperature $T_{A,L}$ of magma A at $x = L$ equal to 1,000 °C, so that the subaxial flow of magma can occur before complete solidification, whereas $T_{A,0}$ is taken as the olivine crystallisation temperature observed over Iceland and the Azores (Figs 3 and 5*b*). As a first approximation, we assume that T_A also varies linearly with x, that is,

$$T_A = T_{A,0} + bx \quad (5)$$

where the slope $b = T_{A,0} - T_{A,L}/L$. Combining equations (2), (4) and (5), we then obtain:

$$T_M = T_{A,0} + \left(\frac{T_B - T_{A,0}}{L} - b\right)x + \frac{bx^2}{L} \quad (6)$$

A broad minimum for T_M is predicted at $x = bL - T_B + T_{A,0}/2b$. The minimum occurs at $L/2$, if $T_B = T_{A,0}$ (Fig. 5). Higher order forms for $T_A(x)$ shown in Fig. 5 can displace the minimum to $x < L/2$, but do not really change the broad form of the minimum between $0 \leq x \leq L$ (Fig. 5). It seems, therefore, that the predictive curves based on such a mixing model cannot readily account for the rapid rise observed in T_M with x past the minimum (Fig. 5). The observed minimum south of Iceland and the Azores is narrower and more localised than the broad minimum predicted by a mixing model constructed on the basis of the boundary conditions imposed by the isotopic and La/Sm gradients.

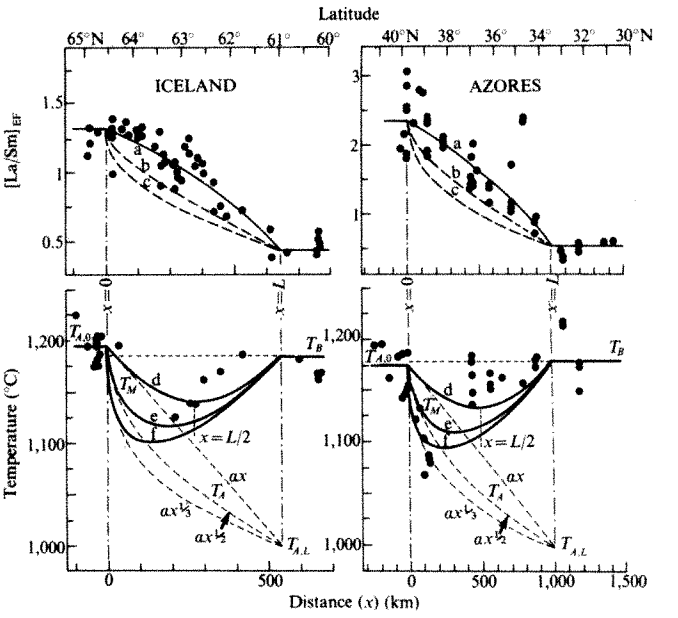


Fig. 5 Top, comparison of actual La/Sm variations with theoretical curve based on a binary magma mixing model assuming: curve a: $S = 1 - x/L$; curve b: $S = 1 - (x/L)^{1/2}$; curve c: $1 - (x/L)^{1/3}$. Average La and Sm content of end member melts are in p.p.m.: Iceland—$La_A = 6.9$; $La_B = 1.44$, $Sm_A = 3.7$; $Sm_B = 2.3$. Azores—$La_A = 15.0$, $La_B = 2.3$, $Sm_A = 4.5$, $Sm_B = 3.1$. Bottom, comparison of: predicted temperature of mix-magma M, based on the binary magma mixing model (lines), with calculated temperature based on Mg partitioning between olivine and glass (●). The following $T_A(x)$ functions (light dashed lines) were used to calculate curves T_M (heavy lines) for models d, e and f, respectively: Model d: $T_A = T_{A,0} + bx$ with $b = (1{,}000 - T_{A,0})/L$; Model e: $T_A = T_{A,0} + bx^{1/2}$ with $b = (1{,}000 - T_{A,0})/L^{1/2}$; Model f: $T_A = T_{A,0} + bx^{1/3}$ with $b = (1{,}000 - T_{A,0})/L^{1/3}$ All T_M models (d, e and f) were computed assuming $S = 1 - (x/L)$ correspond with solid line a on top diagram; and $T_{A,L} = 1{,}000$° C. Values for other parameters are: $L = 540$ km, $T_{A,0} = 1{,}195$ °C and $T_B = 1{,}184$ °C in the case of Iceland, and $L = 1{,}000$ km, $T_{A,0} = 1{,}175$ °C and $T_B = 1{,}178$ °C in the case of the Azores.

Conclusions

We conclude that subaxial flow of plume-derived magma and its progressive mixing with LILE-derived materials is apparently an oversimplification and cannot by itself account for the observed width of these two temperature minima. Additional tectonic

controls need to be invoked to explain the shape of these two minima. These include: (1) close-spaced fracture zone fabric near hotspots exposing magma to colder fault planes and further enhancing circulation of seawater within the crust and thus the efficiency of heat loss from the subaxial magma chamber, or (2) lack of a steady-state regime in the subaxial magma chamber beneath these two ridge segments, perhaps signalling recent rift jumping, or tectonic readjustment; or perhaps a simpler explanation (3) the two large temperature minima may reflect the temperature and composition of melts within two large subaxial magma chambers associated with the Iceland and Azores Plateaus.

We consider the cooling effects caused by close-spaced fracture zone fabric or the presence of a large magma chamber as the most probably dominant factors in producing the observed temperature minima south of Iceland and the Azores, as our empirical model alone, involving cooling by subaxial flow and mixing, provides too broad a minimum. However, this does not mean that subaxial flow and mixing does not occur, as it is also suggested by previous petrographic and geochemical evidence. We also suggest that the lower quenching temperatures of mid-ocean ridge basalts erupted near major fracture zones as compared with magma erupted along unfractured ridge segments may be caused by exposing magma chamber to colder fault planes due to seafloor spreading and by enhancing seawater circulation. The study of basalts collected at close intervals near other fracture zones and hotspots coupled with detailed state-of-the-art geophysical surveys should help in testing our inferences further.

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Mesozoic ophiolite and blue amphibole on Timor and the dispersal of eastern Gondwanaland

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A dismembered metamorphosed ophiolite on Timor is connected with Jurassic rifting of Australia's northwestern margin. The rift developed into an ocean basin which carried a microcontinental block northwards. During the middle to late Cretaceous the block accreted to South-east Asia and experienced a low grade metamorphism which generated crossitic amphibole.

IT is generally accepted that Timor is located in a collision zone between South-east Asia and the Australian continent, which separated from Gondwanaland during the Mesozoic. However, the place of origin of the different formations and tectonic units of Timor has been widely debated. Three principal models have been proposed that account for the tectonic development of Timor in radically different ways. Hence, its structure is regarded either as an imbricate subduction mélange of Australian components (the imbricate model)[1], a series of block-faulted Australian formations with surficial gravity slides (the autochthon model)[2], or as a number of overthrusted South-east Asian units on para-autochthonous Australian rocks (the overthrust model)[3].

These interpretations have not given adequate consideration to the importance of radiolarian cherts, a suite of metamorphosed igneous rocks, and reports of blue amphibole on Timor: these key elements can unravel the tectonic history of the island. A reappraisal of such data is used here to develop a new model.

Stratigraphic units and their relationships

The Lolotoi Unit is one of the main structural components of Timor. It comprises a diverse assemblage of ?Precambrian to Miocene age rocks[3]. The unit includes the Palelo Group and the Lolotoi Complex. The latter replaces and combines two lithostratigraphic groups—the crystalline schists and ophiolite–spilite complex (Fig. 1). These informal terms are used here.

The crystalline schists are divisible into a low grade younger component and a higher grade older component[4–6]. The older element is polymetamorphic and dominated by primary amphibolite facies assemblages. Semi-pelitic gneisses can contain a kyanite–staurolite–garnet paragenesis[7], typical of intermediate pressure and temperature conditions of regional metamorphism. The monometamorphic younger component is characterised by greenschist facies and lower grade mineral assemblages. Relict clastic and igneous minerals and textures are frequently preserved in the least altered rocks[4]. Typical lithologies include Mn-rich metachert, greenstone (such as spilite) and sericitic phyllite. The older component shows abundant evidence of widespread greenschist facies retrogression[8], possibly associated with the metamorphism which affected the

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low grade rocks[6]. In several areas of Western Timor the crystalline schists are overlain unconformably by the Palelo Group (Fig. 1). This comprises a lower series which contains radiolarian cherts and altered basaltic rocks, and an upper part (the Haulasi Formation) dominated by flysch-type deposits.

There is sufficient field and petrographic evidence to demonstrate that the low grade crystalline schists of the Miomaffo district of Western Timor[4] are closely related to the lower Palelo, and that the ophiolite–spilite complex is broadly coeval with both. Spilite and keratophyre occur in the lower Palelo and low grade component of the Lolotoi Complex. Volcanic breccias near the base of the lower Palelo contain clasts of metamorphosed and strongly deformed basalts, presumably eroded from the low grade rocks, together with less-deformed and metamorphosed basalt clasts derived from interlayered flows. The lower Palelo itself is weakly metamorphosed and often strongly deformed[4]. Igneous activity and deposition were evidently broadly contemporaneous with, but outlasted by, metamorphism and deformation.

Cherts of the lower Palelo contain radiolaria of late Jurassic to early Cretaceous type, while limestones near the top of the sequence contain foraminifera of Aptian to Turonian age[9]. The occurrence of apparently related igneous rocks within the lower Palelo, ophiolite–spilite complex and low grade crystalline schists implies a Jurassic to Cretaceous age for the entire section.

The ophiolite–spilite complex includes gabbroic rocks and extensive masses of ultramafics, which show varying degrees of deformation and metamorphism. Published evidence suggests that serpentinite underlies the crystalline schists in the Mutis[7,10] and Miomaffo[4] districts. In other areas, however, serpentinite is intercalated with, or rests on top of, the schists[8,11]. All cases lack a high temperature thermal aureole and the ultramafics are generally strongly deformed. The ophiolite–spilite complex is older than the unmetamorphosed Haulasi Formation, in which clasts of the complex are found[7]. The tectono-metamorphic episode which affected the complex and the lower Palelo is thus bracketed between the Upper Jurassic and Upper Cretaceous.

The Boi Massif of Western Timor is composed of biotite–garnet–gneiss, serpentinite, metagabbro and amphibolite gneiss[11]. Homogeneous metagabbro is transitional between an interlayered sequence of amphibolite and metagabbro. This unit contains concordant intercalations of high-grade metasedimentary gneiss. Thin sections of the gneiss show sillimanite pseudomorphing biotite, and polygonal aggregates of cordierite and hercynite replacing biotite, garnet and sillimanite. The mineral assemblages record a temperature increase to around 700 °C and a pressure decrease equivalent to an uplift of over 7 km (ref. 11). I consider that intrusion of a gabbroic mass caused a thermal overprint during uplift of the lower continental crust. The pelitic gneiss was already regionally metamorphosed, but emplacement of the gabbro preceeded further metamorphism and deformation. If the gabbro is related to the ophiolite–spilite complex, this provides additional evidence for Jurassic–Cretaceous 'orogenic' events within the Lolotoi Unit.

The Haulasi Formation is a late Cretaceous to Palaeocene flysch sequence which contains clasts derived from the lower Palelo, crystalline schists and ophiolite–spilite complex[4,7]. There are also fragments of vesicular basalt which resemble alkaline lavas in the Permian Maubisse Formation of Timor.

The Lolotoi Complex and Palelo Group are intimately related. If they formed part of the northwestern margin of Gondwanaland, it should be possible to correlate their development with the geology of subsurface rocks to the south of Timor, and with Australian shelf and rise deposits which outcrop on the island. Recent marine and on-land investigations have shown that Australian continental crust underlies the Timor Sea[12] and most, if not all, of Timor[13]. Published accounts[14–16] of the Bonaparte Gulf and Wharton basins (Fig. 2) are used as a basis for the following discussion.

Pre-middle Jurassic correlation

It is generally agreed that the northwestern Australian shelf became fragmented by rifting and major uplift in the late Carboniferous. This exposed Precambrian rocks, on which were deposited late Carboniferous to late Permian sediments. This sequence has been considered as a lateral equivalent of the 'Permian' Aileu and Permian Maubisse formations of Timor[17]. The latter contains rift-type alkaline basalts[18] which are similar to those in the Australian facies Cribas Formation of Timor[17]. It has been suggested that reefal carbonates of the Maubisse Formation might have developed on a basement high near the northern margin of Australia, this basement being represented by the Lolotoi Complex[17] (probably the older component of the crystalline schists).

In the Bonaparte Gulf Basin, late Carboniferous to early Triassic sedimentation is characterised by a series of marine transgressions and regressions. On Timor, the reported interlayering of the Maubisse Formation with Australian continental

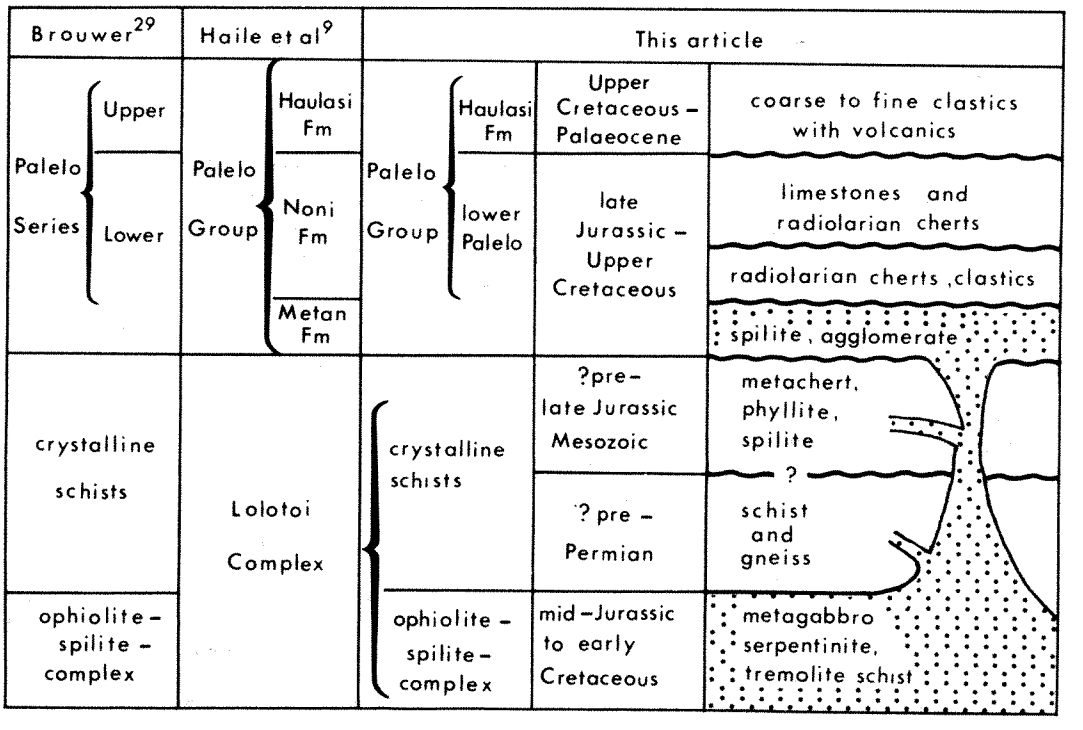


Fig. 1 Nomenclature and stratigraphy of pre-Eocene formations in the Lolotoi Unit.

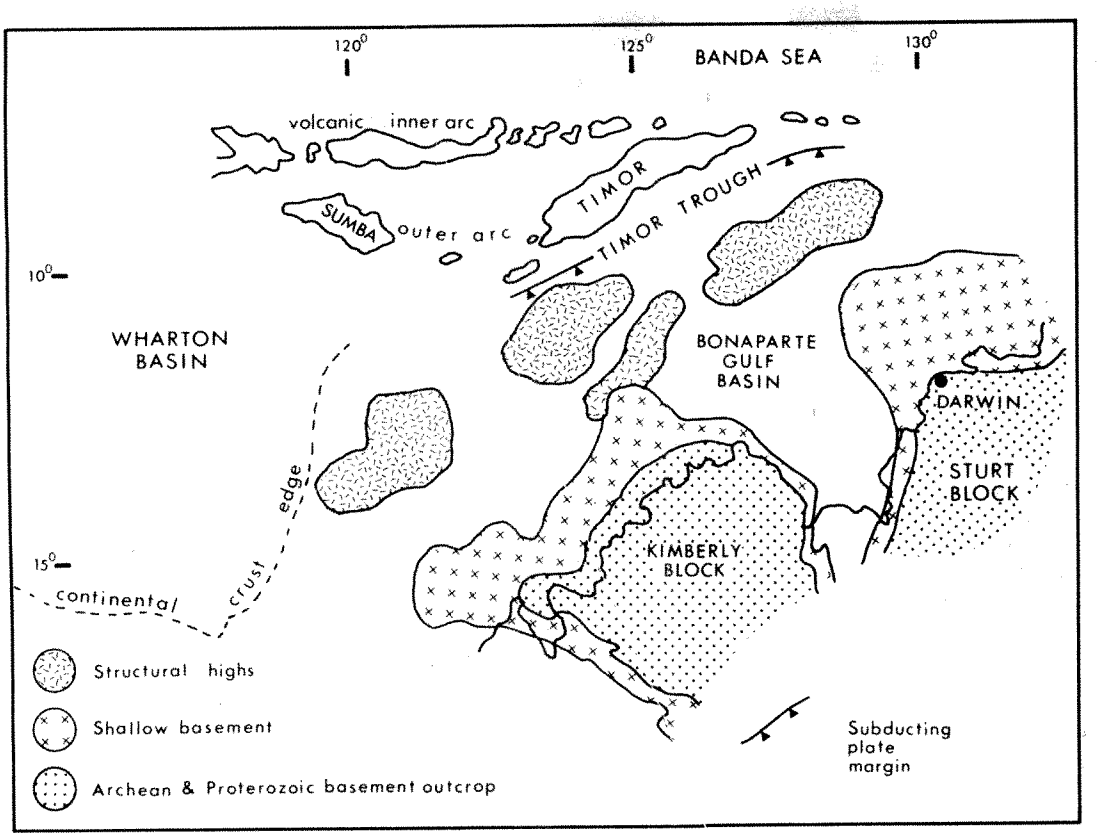


Fig. 2 Location map. Pre-Permian basement ornamented. Modified after Kaman-Kayes and Meyerhoff[19].

rise deposits (Cribas Formation)[17] is explicable in terms of these relative changes in sea level, which must have caused lateral facies changes. If these units intertwine it would prove that the Maubisse Formation was deposited on the northern margin of eastern Gondwanaland, and is not an allochthonous unit derived from South-east Asia as claimed by supporters of the overthrust model[3].

Middle Jurassic to middle Cretaceous correlation

In the Bonaparte Gulf Basin middle Jurassic sedimentation was interrupted by a major regional epeirogeny. Precambrian basement became exposed in highs of the horst and graben structures which developed before recommencement of deposition in the Upper Jurassic. Extensive areas of basaltic rocks on the north-west shelf[19] might be related to the formation of new ocean crust (the Indian Ocean)[15] and to uprisen mantle identified under the Bonaparte Gulf Basin[20]. It can be argued that part of the ophiolite–spilite complex represents middle to late Jurassic igneous activity associated with rifting of the Australian continental margin. Gabbroic magma invaded continental basement which was being uplifted and eroded as part of the rifting process.

In the Bonaparte Gulf Basin, Precambrian blocks supplied detritus until the Neocomian, when all traces of a northerly and northwesterly sediment source were eliminated, presumably by seafloor spreading[15,16]. The landmass which supplied the sediment has been identified as the submarine ridge which underlies Sumba and the islands towards Timor[20] (Fig. 2). Therefore, the rift possibly extended as far as Timor, and perhaps beyond. Rapid subsidence and a marine transgression in the Aptian allowed deposition of bathyal radiolaria- and glauconite-rich sediments in the Timor Sea region[15]. They rest uncomfortably on Upper Jurassic to Precambrian rocks, just as bathyal radiolarian cherts of the lower Palelo rest uncomforbably on the Lolotoi complex.

Thus far, the autochthon model[2,17] correlates well with the stratigraphic and structural development of Australia's north-western margin, but during the Cretaceous the two begin to diverge[21].

Middle Cretaceous to Palaeocene developments

Oceanic crust (the Indian Ocean) formed as a result of rifting which affected the continental crust right down the western side of Australia[22]. A separate plate, the Wharton Basin (Fig. 2), closed eastwards to a pole of rotation north of Timor[14]. This developing ocean basin must have transported rifted continental crust (the Western Landmass) northwards towards South-east Asia. It is inferred that this landmass included the Lolotoi Complex, and perhaps the Aileu and Maubisse formations. Using different criteria Barber[21] and Johnston[23] have proposed that a microcontinental block separated from Australia in the region of Timor, and accreted to South-east Asia during the Cretaceous.

The Haulasi Formation does not contain clasts derived from the Australian shelf and rise deposits of Timor[4,5]. This implies that throughout the Upper Cretaceous and Palaeocene the Lolotoi Unit was nowhere near the northern margin of Australia. A significant proportion of the fragments in the Haulasi Formation was derived from andesitic volcanics, and there are interlayered tuffs[4]. The minerals are often unaltered, in contrast with volcanic, schist and chert fragments eroded from the lower Palelo and Lolotoi Complex. The andesitic volcanics might therefore be products of middle to late Cretaceous subduction on the southern margin of South-east Asia.

Blue amphibole has been reported from Timor[4,5,7,18] and neighbouring Leti[24]. Petrographic descriptions of basaltic rocks from the Permian Maubisse Formation[18] and the ophiolite–spilite complex[7] show unequivocally the presence of crossite in these rocks. Crossite occurs as rims around, or as parallel intergrowths with, secondary green amphibole and primary pyroxene and brown amphibole. A crossite-bearing greenschist was found as a clast in the Haulasi Formation of the Miomaffo region[4]. If the amphibole has been identified correctly it implies that crossite grew before deposition of the Upper Cretaceous deposits, and after the formation of the ophiolite–spilite complex, which also contains crossite. This metamorphism possibly represents relatively high pressure–intermediate temperature conditions which could have been realised during accretion of the rifted crustal block against the leading margin of

South-east Asia. Subduction might have been short-lived so that isothermal surfaces were not sufficiently depressed to generate typical blueschist facies conditions. The postulated metamorphic episode is broadly contemporaneous with a major orogenic phase which produced greenschists on many other islands of the Outer Banda Arc[25]. The 'subducted' block was subjected to uplift and erosion, supplying detritus for the Haulasi Formation. This probably formed a prograding wedge of flysch deposited in the arc–trench gap[21].

Palaeocene to Recent developments

In several massifs of Western Timor, Eocene strata rest unconformably on the Palelo Group and/or the Lolotoi Complex[4,5,8,26,27]. The unconformable relationships are possibly connected with isostatic adjustments which probably attended cessation of seafloor spreading in the Wharton Basin (about 55 Myr ago[14]), and tectonic activity associated with back-arc rifting which generated the Banda Sea[28]. These events roughly coincide with the final separation of Australia from Antarctica during the Eocene, and with the culmination of a late Mesozoic to early Tertiary orogenic phase identified in the Outer Banda Arc and Eastern Sulawesi[25]. Presumably subduction must have occurred south of the microcontinent to explain the presence of andesitic volcanics in the Eocene formations[4,21]. Andesitic volcanics of Eocene age are also found on Sumba, which probably formed part of the same arc.

The Miocene to Recent history of Timor is well documented, but is open to radically different interpretations[1–3]. The ideas presented here include elements from each of the three main models. Sometime after the Oligocene, Australian continental crust reached the subduction zone. The outcrop trace of the plate boundary is currently located in the axis of the Timor Trough[1] (Fig.2). The Australian shelf and rise deposits which outcrop on Timor have thus been transferred to the overriding plate. Near the south coast of Western Timor, some of these deposits (the Kolbano Unit) are imbricated[3,10]. They form part of an imbricate wedge behind the trench axis, in accordance with the imbricate model[1]. Emplacement of overthrust units[3] from the north of this zone was caused by underthrusting of the advancing margin of Australia. They include units derived from the postulated microcontinent, as well as an olistostrome. Some of these formations originated from Gondwanaland, but the post-lower to middle Cretaceous sediments and volcanics were deposited on the southern margin of South-east Asia and in the ocean basin to the south.

Conclusions

The Lolotoi Unit contains a dismembered metamorphosed ophiolite which formed during the breakup of Australia's northwest shelf during the Jurassic.

A rifted block of continental crust drifted northwards from Australia, and accreted to South-east Asia during the Cretaceous.

Crossitic amphibole grew during low grade metamorphism within the block.

Subduction south of the block generated andesitic volcanics during the late Mesozoic and early Tertiary.

The block rifted from mainland South-east Asia and met with the northwards drifting Australian continent during the Neogene.

Australian deposits were transferred to the overriding plate and some became imbricated, whereas formations closer to the inner arc were emplaced southwards as overthrust units.

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The transforming gene of Moloney murine sarcoma virus

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A cleavage map of the Moloney murine sarcoma viral DNA was constructed and compared with that of a spontaneously occurring deletion mutant. By restriction enzyme analysis, it was shown that a region encompassing over 40% of the viral information was not essential for transformation or rescue of the deletion mutant. The transforming region was further localised by analysis of the transforming activity in tissue culture of isolated restriction fragments of linear double-stranded sarcoma viral DNA. In each case, DNA fragments that retained transforming activity preserved the cell-derived insertion sequences of the viral genome. Moreover, such transformants invariably expressed RNA specific to this region. By these two approaches, it was possible to demonstrate that the transforming region of the viral genome begins very near or within the cell-derived insertion sequences. Thus, the transforming gene of this mammalian sarcoma virus originates from within the mouse cell genome.

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RNA-CONTAINING sarcoma viruses that transform fibroblasts in tissue culture and induce solid tumours *in vivo* have been isolated from several mammalian species. These replication-defective viruses possess some genetic information of the type-C helper leukaemia virus isolated in association with them[1-3]. In addition, they contain non-helper viral sequences that are genetically related to cellular DNA of the species from which the sarcoma virus was derived[3-5]. By heteroduplex and oligonucleotide mapping, as well as by hybridisation techniques, non-helper viral sequences have been localised to a specific region of the sarcoma viral genome[6-8]. Thus, accumulating evidence suggests that mammalian sarcoma viruses may have arisen by recombination of a type-C helper virus with some cellular genetic information.

The replication-defective nature of mammalian sarcoma viruses has made genetic analysis of these viruses very difficult. Thus, it has not been possible to identify the region of the viral genome necessary for malignant transformation. Recently, the application of restriction endonuclease technology has led to generation of a physical map of the Moloney strain of murine sarcoma virus (MSV)[9]. In the present report, we have taken advantage of this biochemical approach to identify portions of the viral genome which can be deleted either in a naturally occurring mutant or in a restriction enzyme cleavage product, without affecting the transforming ability of the genome. By this strategy, it has been possible to identify and map directly the transformation-inducing information of MSV. The present findings have implications concerning the origin of transforming genes of mammalian sarcoma viruses.

Comparison of the physical map of a transforming deletion mutant with parental MSV-124 DNA

The major species of viral DNA in NIH/3T3 cells newly infected with the 124 strain[10] of MSV is a linear double-stranded molecule, postulated to be a complete transcript of MSV RNA[9]. Several restriction endonucleases cut this DNA, and a preliminary physical map of the MSV genome has been constructed[9]. Using the superior Southern technique of direct transfer of DNA fragments from agarose gels to nitrocellulose sheets[11], we refined and extended this map. Linear MSV-124 DNA was purified from infected cells and digested with several restriction endonucleases. The resulting fragments were fractionated on agarose gels, blotted onto nitrocellulose sheets, hybridised to ^{32}P-labelled viral complementary DNA (cDNA) and autoradiographed.

Linear MSV-124 DNA, with a molecular weight (MW) of $3.65 \pm 0.1 \times 10^6$, was cleaved by *Xho*I enzyme (Fig. 1*a*) into two fragments of MWs 2.4×10^6 and 1.25×10^6. Digestion with *Sal*I endonuclease (Fig. 1*b*) generated fragments of MWs 1.85×10^6 and 1.75×10^6. Digestion with *Bgl*II enzyme (Fig. 1*c*) produced two fragments of MWs 2.25×10^6 and 1.35×10^6. *Bgl*I (Fig. 1*d*) cut the DNA into fragments of MWs 2.35×10^6, 1.05×10^6 and 0.25×10^6. *Xba*I digestion (Fig. 1*e*) yielded fragments of MWs 2.4×10^6 and 1.25×10^6. The cleavage products of MSV DNA digested with *Hin*cII, *Hae*II and *Hin*dIII enzymes have been reported elsewhere[9]. More accurate determination of the size of the different fragments by the blotting method showed that *Hin*cII endonuclease cleaved the DNA into fragments of MWs 1.7×10^6, 1.05×10^6 and 0.85×10^6; *Hae*II enzyme cut MSV DNA into three fragments of MWs 2.4×10^6, 0.95×10^6 and 0.3×10^6; finally, *Hin*dIII enzyme cleaved the DNA into fragments of MWs 3.1×10^6 and 0.55×10^6.

The cleavage sites of *Hin*dIII and *Hin*cII enzymes on MSV-124 DNA were orientated with respect to the 3′ and 5′ ends of the viral RNA[9]. By a series of double digestions (not shown), it was possible to order the different cleavage sites of the enzymes above into a precise and detailed physical map of MSV DNA (Fig. 2).

We have recently isolated a series of transforming deletion mutants of MSV-124 following serial passage of the parental virus in mouse cells (E.C., K.C.R. and S.A.A., in preparation). Among the mutants analysed, MSV-124 clone 14 was found to have the smallest genome, composed of approximately 3,600 nucleotides. This viral genome was rescued from nonproductively transformed NRK cells by superinfection with a heterologous helper virus, woolly monkey type-C virus. The rescued virus was then used to infect NRK cells so as to obtain unintegrated viral DNA. The linear form of MSV-124 clone 14 viral DNA (Fig. 1*f*), which has a MW of 2.25×10^6, was analysed for its susceptibility to those restriction enzymes used for mapping the parental viral genome.

*Xho*I, *Sal*I and *Bgl*II enzymes (Fig. 1*g*, *h*, *i* respectively) did not cleave MSV-124 clone 14 DNA, indicating the lack of cleavage sites for these enzymes in the mutant viral DNA. The *Hae*II enzyme (Fig. 1*l*) produced fragments of MWs 1.30×10^6 and 0.95×10^6, whereas *Bgl*I digestion (Fig. 1*j*) generated two

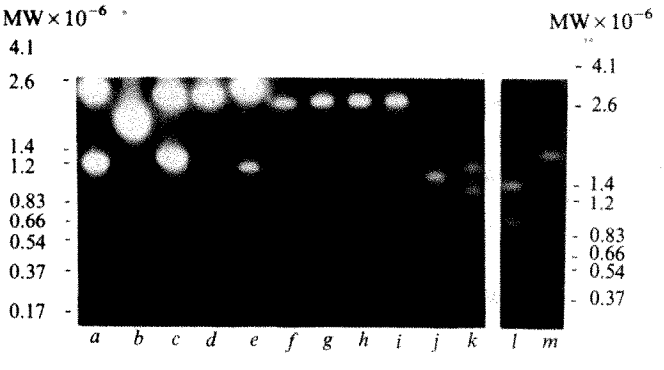


Fig. 1 Analysis of intact and endonuclease-digested MSV-124 and MSV-124 clone 14 DNAs. Linear MSV DNA was obtained from NIH/3T3 cells infected with the 124 strain of MSV or from NRK cells infected with MSV-124 clone 14. About 14 h after infection Hirt supernatant DNA was prepared by a modification of the Hirt procedure[28], and linear MSV DNA was further purified by sucrose gradient centrifugation[9]. Aliquots (2-μg) of MSV-124 DNA corresponding to the amount extracted from 5×10^6 cells, were incubated with endonucleases *Xho*I, *Sal*I, *Bgl*II, *Bgl*I and *Xba*I (lanes *a–e*, respectively). Similarly, 10-μg aliquots of MSV-124 clone 14 DNA corresponding to the amount extracted from 20×10^6 cells were sham treated (*f*) or incubated with enzymes *Xho*I, *Sal*I, *Bgl*II, *Bgl*I, *Xba*I, *Hae*II and *Hin*dIII (lanes *g–m*, respectively). Digestion buffers for *Hin*dIII and *Hae*II enzymes have been reported elsewhere[9]. *Bgl*I and *Bgl*II reaction mixtures contained 6 mM NaCl, 8 mM MgAc, 10 mM Tris-HCl, *p*H 7.5, 6 mM KCl (60 mM KCl for *Bgl*I), 2 mM dithiothreitol and 100 μg ml^{-1} bovine serum albumin (BSA). Reaction mixtures for *Sal*I, *Xho*I and *Xba*I digestions included 140 mM NaCl, 10 mM Tris-HCl, *p*H 7.9, 10 mM MgAc, 2 mM dithiothreitol and 100 μg ml^{-1} BSA. Digestions were carried out in 0.1 ml with the appropriate amounts of each enzyme (New England Biolabs). Following incubation for 2 h at 37 °C, DNAs were extracted with phenol, concentrated by alcohol precipitation, redissolved in 0.05 ml of 10 mM Tris-HCl, *p*H 7.5, and 1 mM EDTA and loaded on a horizontal slab gel of 1.4% agarose. Reference DNA markers of *Hin*dIII-digested λ phage DNA[29] and *Hae*III-digested ΦX174 phage DNA[30] were electrophoresed in parallel. The reference DNA markers at the left side apply to lanes *a–k*; those at the right side apply to lanes *l*, *m*. Electrophoresis was for 15 h at 30 V and 25 mA in a buffer containing 40 mM Tris-HCl, *p*H 7.7, 5 mM NaAc and 1 mM EDTA. The DNA was blotted from the gel and fixed onto nitrocellulose filters as described elsewhere[31]. The filters were incubated at 65 °C for 2 h with a solution of 3 × SSC, 0.02% BSA, 0.02% Ficoll and 0.02% polyvinylpyrrolidone, and further incubated at 65 °C for 2 h with the same solution supplemented with sheared native and denatured calf thymus DNA (50 μg ml^{-1} each), 0.1% SDS and 1 mM EDTA. The nitrocellulose sheet was then transferred to a hybridisation buffer containing 10^6 c.p.m. ml^{-1} of ^{32}P-labelled MSV(MuLV) cDNA. (This viral probe was prepared by reverse transcription of 30S MSV(MuLV)-124 RNA, primed with calf thymus DNA oligonucleotides. The reaction mixture included 20 mM Tris-HCl, *p*H 7.5, 20 mM KCl, 6 mM MgAc, 1 mM dithiothreitol, 18 μg ml^{-1} actinomycin D, 1 mM each of dATP, dGTP and dTTP, 0.5 mM ^{32}P-labelled dCTP at a specific activity of 2–3,000 Ci mmol^{-1} (Amersham/Searle), 10 μg ml^{-1} viral RNA, 1 mg ml^{-1} calf thymus DNA oligonucleotides[32] and purified avian myoblastosis viral reverse transcriptase. Following incubation for 3 h at 37 °C, the cDNA was deproteinised and treated with alkaline to remove viral RNA.) Following hybridisation at 65 °C for 40 h, the strips were washed for 1 h at 65 °C with five changes of hybridisation buffer without cDNA and then washed for 30 min at 65 °C with a solution of 0.1 SSC, 0.1% SDS. The filters were then dipped in 3 mM Tris at *p*H 9.3 at 4 °C and finally rinsed in 1 M NH_4Ac and dried. Strips were mounted on Whatman paper and subjected to autoradiography (Kodak X-Omat R film) for 24 h at room temperature. The small fragments of MN 0.25×10^6 and 0.55×10^6 in lanes *d* and *m* are difficult to visualise in this figure.

fragments of MW 1.1×10^6, which were indistinguishable in their electrophoretic mobility. These results indicated that the DNA of the deletion mutant retained only one of the two cleavage sites for both *Hae*II and *Bgl*I. Finally, the DNA was cleaved by *Xba*I (Fig. 1*k*) to fragments of MWs 1.25×10^6 and 1.0×10^6, and by *Hin*dIII (Fig. 1*m*) to yield fragments of MWs 1.7×10^6 and 0.55×10^6. Therefore, *Xba*I and *Hin*dIII sites were retained in MSV-124 clone 14 DNA. These series of digestions enabled us to construct the physical map of the deletion mutant and to define precisely the portion of MSV-124 DNA deleted during generation of the mutant (Fig. 2). This segment of 2,200 nucleotides resided between, but did not include, the *Xba*I and the 5′-terminal *Hae*II sites of MSV-124 DNA. The deletion mutant was efficiently rescued and possessed transforming activity of high titre, indicating that the deleted segment of the MSV-124 genome is not required for virus replication or cell transformation.

Identification of infectious MSV DNA

An independent approach for defining the region of the viral genome required for cell transformation involved the use of intact and fragmented viral DNA in transfection assays. First, we investigated whether *in vivo* synthesised MSV-124 DNA possessed biological activity. Low molecular weight DNA extracted from cells infected 14 h earlier with MSV(MuLV) was fractionated by agarose gel electrophoresis. The DNA was eluted from the gel slices and tested for focus-inducing activity by a modification of the Graham and von der Eb transfection method[12]. The transforming activity migrated as a single peak with a mobility slightly greater than that of a 4.1×10^6-MW linear marker DNA (Fig. 3). Thus, the focus-inducing activity in the Hirt supernatant consisted primarily of unintegrated viral DNA in the form of a linear double-stranded molecule of MW $3.7\pm0.2\times10^6$. We also analysed the Hirt supernatant DNA, fractionated by agarose gel electrophoresis, for the presence of infectious MuLV DNA. A single peak of reverse transcriptase-inducing activity was detected in the gel at a position of linear double-stranded DNA of MW $5.9\pm0.2\times10^6$ (Fig. 3), corresponding to the known MW of MuLV DNA[13,14].

Transformation by MSV-124 DNA restriction fragments

To establish the infectivity of DNA fragments and, thus, to localise further the region of the MSV genome responsible for transformation, MSV DNA was digested with *Xho*I, *Bgl*II, *Hpa*I or *Hin*dIII enzymes, and fractionated by agarose gel electrophoresis. DNA fragments were then extracted from the gel, and tested by transfection for transforming activity. The fragments were also subjected to agarose gel electrophoresis and blotting analysis to determine their purity. As shown in Table 1, much of the transforming activity of intact linear MSV-124 was retained after digestion with either *Xho*I or *Bgl*II enzymes. The focus-inducing ability was associated with the *Xho*IA and *Bgl*IIB DNA fragments, respectively (Table 1). Digestion of MSV-124 DNA with two enzymes, *Hpa*I and *Hin*dIII, which cut within the cellular insertion of MSV-124, abolished the transforming activity. As shown in Table 1, no focus-inducing ability was retained by either *Hin*dIIIA or *Hin*dIIIB fragments. Similarly, neither *Hpa*IA nor *Hpa*IB fragments retained biological activity.

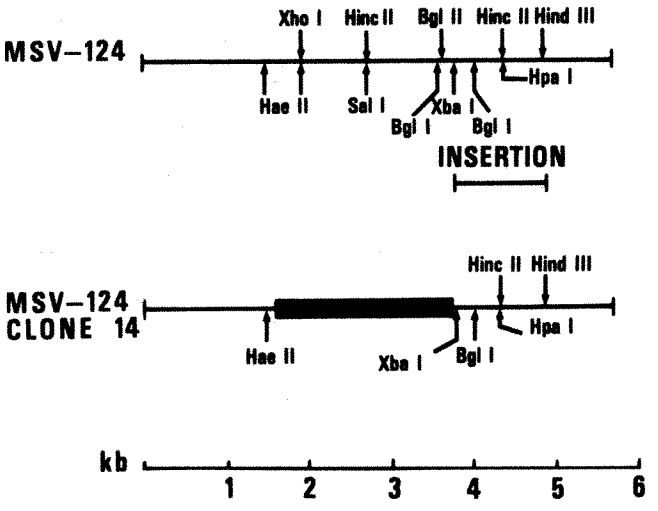


Fig. 2 Physical map of MSV-124 and MSV-124 clone 14 linear DNAs. The right-hand side represents the DNA region corresponding to the 3′ terminus of MSV RNA. The position of *Hpa*I cleavage site has been reported elsewhere[22]. The initial study on the topography of the cellular insertion in the MSV genome was made by Hu *et al.*[6]. The location and size of the insertion as shown here are based on more accurate recent data of G. Vande Woude and colleagues (personal communication) as well as our own results (Tronick *et al.*, in preparation).

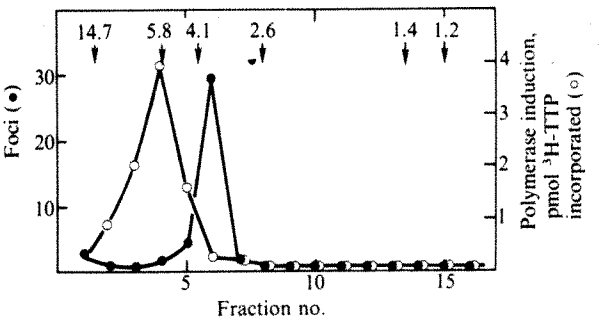


Fig. 3 Agarose gel electrophoresis of infectious MSV and MuLV DNA. Hirt supernatant DNA was prepared from cells infected with MSV-124 as described in Fig. 1. A 35-μg DNA aliquot, corresponding to that extracted from 3×10^7 infected cells, was fractionated by agarose gel electrophoresis as described in Fig. 1. DNA markers of *Hin*dIII-digested λ phage DNA were electrophoresed in parallel and are represented as MW$\times10^{-6}$. The DNA was extracted as described elsewhere[25] from 3-mm gel segments and used to transfect NIH/3T3 cells by modification of the Graham and van der Eb method[12]. Following sterilisation with alcohol, the DNA was redissolved in 0.05 ml of 0.1×SSC and 0.4 ml HEPES buffer, mixed with 25 μg of sheared calf thymus DNA and precipitated by addition of 2 M $CaCl_2$ to a final concentration of 125 mM. After 30 min, the precipitate (0.5 ml) was added to 100-mm Petri dish cultures of NIH/3T3 cells, seeded the previous day with 1×10^6 cells. After 20 min, 10 ml of growth medium were added and the cultures were incubated for an additional 3 h. Dishes were then rinsed twice with growth medium and exposed for 4 min to 5 ml of 15% glycerol in HEPES buffer (N. G. Copeland and G. M. Cooper, personal communication). Finally, the cultures were washed with growth medium and incubated for 10 d, at which time they were scored for focus formation and for release of virion-associated reverse transcriptase activity[33].

Expression of sarcoma viral information in cells transformed by MSV DNA fragments

Transformed foci induced by *Xho*IA or *Bgl*IIB MSV DNA fragments were isolated by the cloning cylinder technique, and several such focus-derived lines were grown to mass culture for further analysis. None of several individual transformants induced by each of the fragments released detectable levels of reverse transcriptase. These findings were not unexpected in view of the fact that these restriction enzymes inactivated the infectivity of helper viral DNA. By morphological criteria, cells transformed by either viral DNA fragment were indistinguishable from transformants induced by intact virus or linear viral DNA. The transformants consisted of round or spindle-shaped, highly refractile cells that grew to saturation densities ranging from 3 to 6×10^5 cells cm^{-2}, compared with 6×10^4 cells cm^{-2} for uninfected NIH/3T3 cells. Moreover, they formed colonies at high efficiency when suspended in soft agar and were tumorigenic in NIH/Swiss mice.

We next investigated cells transformed by individual MSV DNA fragments for expression of sarcoma viral RNA. *Xho*IA and *Bgl*IIB DNA fragments shared a segment of the viral genome, which included the region of MSV RNA previously shown to be MSV specific, that is, not shared with MuLV RNA[6].

Table 1 Transformation of NIH/3T3 cells by MSV DNA restriction fragments

MSV DNA	DNA extending (kilobase pairs)	DNA added (μg per plate)	Foci per plate Expt 1	Expt 2	Expt 3
Intact linear form	0–5.7	30	55	45	65
		15	34	ND	ND
Restriction fragment*					
*Xho*IA	1.9 –5.7	40	ND	25	38
B	0 –1.9	40	ND	0	0
*Bgl*IIA	0 –3.6	40	0	0	0
B	3.6 –5.7	40	11	32	43
*Hpa*IA	0 –4.3	40	ND	0	0
B	4.3 –5.7	40	ND	0	0
*Hin*dIIIA	0 –4.85	40	ND	0	0
B	4.85–5.7	40	ND	0	0

Transfections were carried out in 100-mm Petri dishes and focus formation assayed as described in Fig. 3 legend. ND, Not determined.

* 40 μg of linear viral DNA was digested with restriction endonucleases, deproteinised, precipitated with alcohol and fractionated by agarose gel electrophoresis. Extraction from gel segments was as previously reported[25]. The recovery of the different DNA fragments from the gel was comparable as determined by blotting analysis.

Thus, we reasoned that an MSV-specific cDNA probe could be used to detect transcription of *Xho*IA and *Bgl*IIB DNA. Such a probe was prepared from a DNA transcript of MSV(MuLV) RNA by a series of preparative hybridisations. This involved selection for MSV sequences by hybridisation with MSV(FeLV) RNA followed by selection against sequences annealing to MuLV RNA[15].

To characterise the MSV-specific probe, we determined its ability to anneal to MSV and MuLV sequences, respectively. As shown in Fig. 4*a*, RNA of NIH/3T3 or NRK cells nonproductively transformed by MSV-124 hybridised the entire MSV-specific cDNA, whereas RNA of NIH/3T3 or NRK cells infected with MuLV annealed no more than 6% of the same probe. Further, by analysis of hybridisation of the probe to different MSV DNA fragments (not shown), it was possible to conclude that the MSV-specific cDNA was composed predominantly of sequences included in the region of the genome containing non-helper viral sequences[6]. Finally, the cellular origin of the sequences in the MSV specific cDNA[3,15] was shown by the ability of normal NIH/Swiss embryo cellular DNA to anneal the probe to a large extent with kinetics reflecting single copy representation (Fig. 4*b*).

Cellular RNAs of transformants induced by MSV DNA fragments were next tested for their ability to anneal the MSV-specific cDNA probe. As shown in Fig. 5, RNAs of representative *Xho*IA and *Bgl*IIB transformants hybridised more than 40% and 60% of the probe, respectively, at C_rt values above 7×10^3. Similar results were obtained with each of several individual transformants induced by these two DNA fragments. In contrast, RNA of neither NIH/3T3 cells nor NIH/3T3 cells transformed by another murine sarcoma virus, Kirsten MSV, contained detectable amounts of RNA complementary to the MSV-specific cDNA (Fig. 5). These results establish that cells scored as transformed following transfection with MSV DNA fragments expressed MSV-specific RNA.

Comparison of transformants induced by MSV DNA fragments and intact viral DNA

It is well established that replication-defective mammalian sarcoma viruses can be rescued from nonproductively transformed cells by a type-C helper virus[16–18]. It was of interest to determine whether focus-forming activity could be rescued from cells transformed by MSV DNA restriction fragments. Following MuLV superinfection, none of several individual transformants induced by *Xho*IA or *Bgl*IIB fragments released detectable focus-forming virus (Table 2). In the same conditions, high-titre MSV was released by cells transformed by intact MSV-124 or MSV-124 clone 14 virus, or by linear MSV-124 DNA. The amounts of helper virus produced were comparable for all cell lines tested. These results established that only those transformants induced by the MSV DNA fragments lacked rescuable focus-forming activity.

Some MSV strains code for helper virus *gag* gene products[1,19,20]. By analogy with the avian type-C viral genome[21] the *gag* gene of the mammalian type-C virus is presumed to be localised to the 5′ region of the viral genome. By heteroduplex mapping, MSV has been shown to contain a long region of helper viral sequence homology beginning at the 5′ end and extending approximately 2.25 kilobases[6]. Thus, it is likely that this region contains the helper viral sequences coding for *gag* gene proteins.

To determine whether *gag* gene expression might serve as a useful marker of the intact MSV 124 genome, we analysed several MSV 124 and MSV-124 clone 14 nonproducer transformants for expression of the N-terminal *gag* gene protein, p15 (ref. 19). As shown in Table 2, p 15 was detected at levels of 30–500 ng per mg cellular protein in each virus nonproducer transformant analysed. Similarly, nonproducer transformants induced by linear MSV DNA expressed p15. As the *Bgl*IIB DNA and *Xho*IA fragments originate about 3.6 and 1.9 kilobase pairs from the 5′ terminus of the MSV genome, respectively, it was considered unlikely that transformants induced by these fragments would possess the coding capacity for MuLV p15. As shown in Table 2, none of the transformants induced by *Bgl*IIB or *Xho*IA DNA expressed detectable levels of this virus-coded protein. These findings are consistent with the assignment of transforming activity to *Xho*I and to *Bgl*II DNA fragments other than those encompassing the 5′ terminus of the transforming virus genome.

The transforming gene of mouse sarcoma virus

In the present studies, two independent approaches have been used to localise the region of the Moloney MSV genome

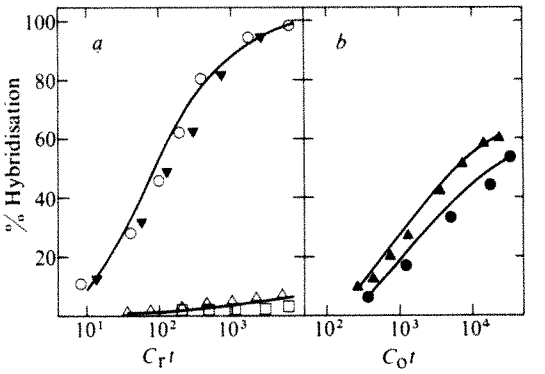


Fig. 4 Characterisation of MSV specific cDNA probe. MSV (MuLV) cDNA was prepared as described in Fig. 1 legend. MSV-specific cDNA was obtained from it by a series of preparative hybridisations in which cDNA sequences unique to MSV were selected by hybridisation with MSV(FeLV) RNA, followed by hydroxyapatite chromatography. Sequences in the cDNA shared with MuLV were removed by two cycles of hydroxyapatite after annealing with MuLV RNA[15]. Cellular RNA was purified by the method of Scherrer[34]. Cellular DNA was prepared as described elsewhere[35], and sheared to a mean size of 6–8S. Unique sequence cellular DNA was similarly prepared and highly reiterated sequences annealing at a C_0t of 50 mol $s\,l^{-1}$ were removed by fractionation on hydroxyapatite. For cDNA–RNA hybridisation, varying amounts of RNA were annealed to 500 c.p.m. of ^{32}P-labelled MSV-specific cDNA in 0.05 ml hybridisation mixtures containing 10 mM Tris-HCl, *p*H 7.5, 1 mM EDTA and 0.6 M NaCl. After incubation for 18–48 h at 68 °C, hybrid formation was determined with S_1 nuclease. cDNA–DNA annealing and hybrid detection were carried out in similar conditions except that the RNA was substituted with cellular DNA at 4 mg ml^{-1}, and the hybridisation was carried out for varying times at 62 °C. *a*, Hybridisation of MSV-specific cDNA by cellular RNAs of MSV-124 transformed nonproducer NIH/3T3 (○); MSV-124-transformed nonproducer NRK (▼); Moloney MuLV-infected NIH/3T3 (△); Moloney MuLV-infected NRK (□). *b*, Hybridisation of MSV-specific cDNA by NIH Swiss mouse embryo DNA (●). The rate of this reaction was compared with the rate of hybridisation of tracer amounts of mouse unique sequence cell ^{3}H-DNA by its homologous unlabelled cell DNA (▲).

involved in transformation. By comparison of the restriction endonuclease map of the viral genome with that of a deletion mutant isolated from the Moloney MSV stock following several cycles of virus passage, a large region encompassing almost 40% of the viral information was shown to be nonessential for transformation or virus replication. The dispensable sequences extend 1.5–3.7 kilobase pairs on MSV-124 DNA. Analysis of the transforming activity of subgenomic DNA fragments isolated following restriction endonuclease cleavage of linear viral DNA revealed that the *Xho*IA and the *Bgl*IIB DNA fragments were highly active. The *Bgl*IIB fragment, which is the smaller of the two, extends 3.6–5.7 kilobase pairs and therefore lacks not only the 5′ terminus, but also about two-thirds of the viral genome. Thus, the evidence obtained by these two approaches fixes the 5′ extent of the maximum information necessary for transformation at or near the *Xba*I site, about 3.7 kilobase pairs from the 5′ terminus.

In contrast to our findings that most of the transforming activity of the viral DNA was preserved after cleavage with *Xho*I or *Bgl*II enzymes, transforming activity was abolished after digestion with *Hpa*I or *Hin*dIII enzymes. As the first enzyme cleaves in the middle and the latter cuts near the 3′ terminus of the cellular insertion sequences, these findings suggest that the information within a large portion of the cellular insertion are essential for transformation.

Andersson *et al.*[22] have recently shown that subgenomic fragments of *in vitro* synthesised MSV DNA retain transforming activity. Their smallest transforming fragment spanned a region of 2.1 kilobase pairs encompassed by the *Sal*I and *Hin*dIII restriction sites. This fragment contains 0.9 kilobase pairs of additional genetic information at the 5′ end compared with the DNA segment shown here to be required for cell transformation. The preservation of transforming activity following *Hin*dIII digestion of the viral genome as reported by Andersson *et al.*[22] is in contrast to our results. The reasons for this discrepancy are unclear. We have also encountered at low frequency transformants induced by MSV DNA fragments generated by digestion with an enzyme, *Hin*cII, which cuts the viral genome within the insertion sequences. However, when these transformants were analysed, they were found to express viral RNA complementary to portions of the viral genome in addition to the *Hin*cIIB transforming fragment (spanning 2.7–4.4 kilobase pairs). We were thus unable to conclude that these transformants were induced by the *Hin*cIIB fragment alone.

Table 2 Properties of NIH/3T3 cells transformed by MSV DNA restriction fragment

NIH/3T3 cell line	Sarcoma virus rescue* No. positive/ No. tested	Sarcoma virus rescue* Range (FFU ml^{-1})	M-MuLV p15 expression† No. positive/ No. tested	M-MuLV p15 expression† Range (ng per mg cell protein)
Transformed by MSV-124 virus	4/4	$10^{5.4}$–$10^{5.8}$	4/4	30–500
MSV-124 clone 14 virus	9/9	$10^{5.4}$–$10^{5.9}$	3/3	30–200
Linear DNA MSV-124	5/5	$10^{5.6}$–$10^{5.9}$	2/2	50–100
*Bgl*IIB DNA MSV-124	0/5	$<10^{0}$	0/5	<5
*Xho*IA DNA	0/2	$<10^{0}$	0/2	<5
Uninfected	—	$<10^{0}$	—	<5

* Around 10 days after infection of each cell line with 10^5 XC plaque-forming units (PFU) of MuLV, 0.5 ml of tissue culture fluids were assayed for focus-forming units (FFU)[1] and XC PFU[26] on NIH/3T3 cells as previously described.

† Cultures were sonicated for 1 min in the presence of 10 mM Tris-HCl, *p*H 7.8, containing 200 mM NaCl, 0.05 mM EDTA, and 1% (v/v) Triton X-100. The soluble protein fraction, obtained from the supernatant of a 150,000*g* centrifugation, was tested at twofold serial dilution in an homologous Moloney-MuLV p15 radioimmunoassay as previously described[19]. Levels of viral protein expression were calculated on the basis of (1) the amount of cellular protein, determined according to Lowry *et al.*[27], and (2) the sensitivity of the p15 immunoassay.

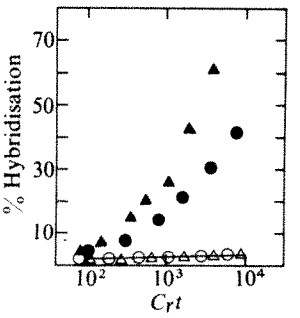


Fig. 5 Expression of MSV-specific RNA in cells transformed by MSV DNA fragments. Hybridisation was carried out as described in Fig. 4 legend. MSV-specific cDNA was hybridised to cellular RNA from uninfected NIH/3T3 cells (△), NIH/3T3 cells transformed by the *Bgl*IIB MSV DNA fragment (▲), NIH/3T3 cells transformed by the *Xho*IA MSV DNA fragment (●), and NIH/3T3 cells transformed by Kirsten MSV (○).

Transformants induced by *Xho*IA and *Bgl*IIB fragments were shown to express RNA specific to the region of the MSV genome from which the fragments were derived. Moreover, the transformants tested lacked the ability of the parental sarcoma virus to express translational products of the 5′ region of the viral genome. The lack of recovery of focus-forming activity from transformants induced by DNA restriction fragments provided even more striking evidence of the phenotypic differences between these cells and transformants induced by virus or intact linear viral DNA. The lack of rescuability of focus-forming activity from transformants caused by MSV DNA fragments remains to be resolved, but may reflect a requirement for preservation of termini of the viral genome for efficient virus rescue and for biological activity of the viral RNA. This possibility is favoured by our findings that the deletion mutant, which lacked a large portion of viral information but preserved both termini, was efficiently rescued and demonstrated transforming activity of high titre.

It has been shown that the genomes of replication-competent type-C viruses of both avian and mammalian origin possess leader sequences originating at their 5′ terminus. These leader sequences become associated with messenger RNAs originating from distal portions of the viral genome[23,24]. Neither of the *Bgl*IIB or *Xho*IA MSV DNA fragments with transforming activity contained 5′-terminal sequences of the parental viral genome. It can be argued that MuLV DNA present in the MSV DNA preparations may have acted to complement transformation by MSV DNA. However, we consider this highly unlikely as MuLV DNA represented only a small fraction (5%) of the viral DNA. Moreover, such MuLV DNA would have to co-migrage with each of the different-sized MSV-transforming DNA fragments used for transfection. Presumably, therefore, viral RNAs transcribed from the transforming MSV DNA fragments of *Bgl*II or *Xho*IA either do not contain leader sequences or derive such a sequence from cellular DNA.

The present studies demonstrate that the helper viral sequences extending from the 5′ end of the cellular insert to the 5′ end of the MSV genome were not essential for transformation. In contrast, cleavage within the cellular insertion inactivated transforming activity. These findings establish that viral information required for transformation by this mammalian sarcoma virus originates from within the cellular genome. By recombinant DNA techniques, it should be possible to obtain a homogeneous population of MSV DNA molecules for further analysis of these sequences. Such techniques should also allow the isolation, amplification and sequencing of the cellular gene homologous to the transforming region of MSV. These studies may make it possible to determine whether the MSV-transforming gene originates from an altered cellular gene or from one that is normally only expressed before the development of the fully differentiated state.

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letters

Does PSR0329+54 have companions?

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An extensive pulse timing programme has been carried out over the past 10 years for several pulsars[1–5]. The timing observations revealed a wide range of different effects such as glitches, noise in the pulsar rotation and unexpectedly large second time derivatives of the rotation frequency. We suggest here that these unexpectedly large values of $\ddot{\nu}$ may be caused by a binary motion with an orbital period, P_b, longer than the span of timing observations. Even a distant planet of a relatively low mass ($100\,M_\oplus$ for $P_b = 50$ yr) would influence $\ddot{\nu}$. In the case of PSR0329+54, besides a large $\ddot{\nu}$ term, a quasi-sinusoidal modulation shows up in timing residuals. This 3-yr periodicity may be caused by: (1) a change in pulse shape, (2) precession of a magnetic dipole axis, or (3) a small planet ($m = 0.06 - 0.57\,M_\oplus$) orbiting the pulsar.

All known pulsars are slowing down at a rate conveniently described by the braking index $n = \nu\ddot{\nu}/\dot{\nu}^2$, where ν is the pulsar frequency. All models of energy loss proposed so far give $n \sim 3$. Using the observed values of ν and $\dot{\nu}$ and assuming $n = 3$ it is easy to estimate $\ddot{\nu}$. It turns out that $\ddot{\nu}$ should be very small (except for the Crab pulsar) and therefore the pulse number at time t can be approximated by

$$N(t) = N_0 + \nu t + \tfrac{1}{2}\dot{\nu}t^2 \qquad (1)$$

However, unexpectedly large values of $\ddot{\nu}$ have been measured for 11 pulsars observed at Arecibo[1], and Helfand *et al.*[5] have discussed two other pulsars (PSR0611+22, PSR0823+26) belonging to this class. In addition, the residuals $N_{obs} - N(t_{obs})$ shown for PSR0329+54 and PSR1508+55 (refs 5, 6) also indicate the presence of the large $\ddot{\nu}$ term. Table 1 lists all these pulsars. It also includes the Crab pulsar, PSR0531+21, for which $\ddot{\nu}$ is large because of its very rapid slowdown.

Here we discuss some features of the timing data for PSR0329+54. The barycentric arrival times covering 8 yr of pulsar observations[5] at National Radio Astronomy Observatory and Five College Radio Astronomy Observatory were kindly supplied by Dr D. J. Helfand.

To determine $\ddot{\nu}$ we take

$$N(t) = N_0 + \nu t + \tfrac{1}{2}\dot{\nu}t^2 + \tfrac{1}{6}\ddot{\nu}t^3 \qquad (2)$$

The values of $\ddot{\nu}$ calculated from data covering the first 5, 6, 7 and whole 8 yr of observations are respectively $\ddot{\nu} \times 10^{25} = 0.66, 0.64, 0.48$ and $0.55\ \mathrm{s}^{-3}$. For the last 5 and 6 yr one gets $\ddot{\nu} \times 10^{25} = 0.94$ and $0.51\ \mathrm{s}^{-3}$. The value of $\ddot{\nu}$ does not depend strongly on the set of data. As our sets of data overlap, this cannot be treated as proof that the effect is a real one. In fact, it has been suggested[5,7,8] that the observed values of $\ddot{\nu}$ can result from a noise-type variation in the pulsar rotation. A random-walk type phenomenon in the phase, frequency and/or frequency derivative may lead to large values of $\ddot{\nu}$ when the span of timing data has a finite length.

To check the value of $\ddot{\nu}$ and to find whether it is a real effect, it is derived from independent sets of data; noise processes should give a normal distribution around $\ddot{\nu} = 0$. This procedure, however, cannot be applied to PSR0329+54 at present. A ratio of the change in the pulsar phase caused by the $\ddot{\nu}$ term after time Δt, $\Delta N = \ddot{\nu}(\Delta t)^3/6$, to the measurement uncertainty of the phase determination, $\Delta N = 7 \times 10^{-4}$, is poor for short Δt, hence fits over short spans give meaningless values of $\ddot{\nu}$ because an error assigned is of the order of the fitted value. Moreover a periodic component present in residuals (Fig. 1) strongly influences $\ddot{\nu}$ derived from spans shorter than ~5 yr. However, this analysis could be done successfully for pulsars with a larger $\ddot{\nu}$ term such as PSR0823+26. Unfortunately, at present we do not have data for other pulsars with large $\ddot{\nu}$.

If the large value of $\ddot{\nu}$ does represent a real property of some pulsars then one also has to accept large and sometimes negative values of the braking index as a real effect. Therefore, a

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Table 1 Pulsars with known $\ddot{\nu}$

PSR	Interval fitted (days)	ν (s^{-1})	$\dot{\nu}$ (s^{-2})	$\ddot{\nu}$ (s^{-3})	Braking index*	Refs
0329+54	2,858	1.400	−4.02 E-15	5.55 E-26	(4.81±0.18) E+03	This work
0531+21	1,875	30.206	−3.86 E-10	1.23 E-20	2.515±0.01	4
0540+23	1,367	4.066	−2.55 E-13	3.95 E-25	(2.47±1.02) E+01	1
0611+22	1,584	2.986	−5.31 E-13		3.5 E+02	5
0823+26	1,833	1.884	−6.06 E-15		−1. E+04	5
0950+08	1,562	3.952	−3.58 E-15	−1.70 E-25	(5.26±0.76) E+04	1
1508+55	2,080	1.352	−9.20 E-15		3.25 E+03	6
1541+09	1,668	1.336	−7.68 E-16	−1.12 E-25	−(2.53±0.42) E+05	1
1604−00	1,613	2.371	−1.72 E-15	2.31 E-26	(1.85±0.72) E+04	1
1859+03	1,226	1.526	−1.74 E-14	1.10 E-24	(5.53±0.50) E+03	1
1907+00	780	0.983	−5.33 E-15	−2.49 E-25	−(8.61±1.42) E+03	1
1907+10	993	3.526	−3.28 E-14	−1.69 E-24	−(5.55±0.40) E+03	1
1915+13	1,602	5.138	−1.90 E-13	−6.44 E-25	−(9.15±1.44) E+01	1
1929+10	1,374	4.415	−2.25 E-14	−3.18 E-25	−(2.76±0.26) E+03	1
2002+31	1,606	0.474	−1.67 E-14	7.13 E-26	(1.21±0.90) E+02	1
2020+28	1,637	2.912	−1.61 E-14	1.03 E-25	(1.16±1.80) E+03	1

* An error quoted corresponds to two standard deviations in $\ddot{\nu}$.

theoretical explanation of this phenomenon is urgently needed.

The large value of $\ddot{\nu}$ derived from the data covering a finite span of time could result from: (1) long time scale irregularities of the pulsar rotation, or (2) from external perturbations such as orbital motion of the pulsar. Irregularities of rotation ('glitches') were observed for several pulsars but their characteristic relaxation times were not longer than a few hundred days. In the models of glitches considered so far it is assumed that the irregularities are caused by a sudden change in the moment of inertia of the neutron star[9,10]. In those models $\ddot{\nu}$ can be large but must be positive during the time of relaxation. Such behaviour was observed for the Vela pulsar after the glitch of September 1975 (ref. 11). For PSR0329+54 the large value of $\ddot{\nu}$ can be explained by this model if the glitch occurred before the discovery of the pulsar and if the relaxation time was longer than the time span of the data. However, it is not possible to say when such a glitch might have occurred. Note that the negative values of $\ddot{\nu}$ observed for some pulsars cannot be explained in this way. Another explanation of this phenomenon is due to Gullahorn and Rankin[12]. They suggest the long time scale (10^2–10^4 yr) variations in the pulsar braking torque. These variations, caused by varying interaction between pulsar and the medium in its vicinity, might give large values of $\ddot{\nu}$ of both signs.

The orbital motion of a pulsar with an orbital period longer than the span of timing observations is very difficult to detect because the effects caused by this motion are masked by the slowdown of the pulsar[13]. Even if ν and $\dot{\nu}$ are strongly influenced by such a motion their analysis cannot show the presence of a binary system unless $\dot{\nu} > 0$ (refs 13, 14).

This orbital motion can be detected more easily, even for a much lighter companion, by looking at higher derivatives of ν which can be drastically influenced by the orbital motion. Let us consider a pulsar moving on a circular orbit. The distance to the pulsar is $d = d_0 + A \sin(\omega t + \phi_0)$ with

$$A = \left[\frac{G}{\omega^2 (M+m)^2}\right]^{1/3} m \sin i,$$

M and m being the mass of the pulsar and its companion respectively. Suppose that during the period of observations $\omega t + \phi_0$ does not change significantly. If $\phi_0 \approx 0$ or π then $d - d_0$ can be approximated by $\pm A(\omega t - \frac{1}{6}\omega^3 t^3)$; if $\varphi_0 \approx \pi/2$ or $3\pi/2$ then $d - d_0 \approx \pm A(1 - \frac{1}{2}\omega^2 t^2)$. Thus, in the most favourable configurations the maximal contributions to ν, $\dot{\nu}$ and $\ddot{\nu}$ are given by $\Delta\nu = \pm\nu A\omega/c$, $\Delta\dot{\nu} = \pm\nu A\omega^2/c$, $\Delta\ddot{\nu} = \pm\nu A\omega^3/c$. If, for example, $P_{\rm orb} = 2\pi/\omega = 50$ yr, $M = 1M_\odot$, $\sin i = 1$ then for $m \ll M$ one gets $A = 6.1\times10^8\ (m/M_\oplus)$ cm, $\Delta\nu/\nu = \pm 8.1\times10^{-11}\ (m/M_\oplus)\ s^{-1}$, $\Delta\dot{\nu}/\nu = \pm 3.2\times10^{-19}(m/M_\oplus)\ s^{-2}$ and $\Delta\ddot{\nu}/\nu = \pm 1.3\times10^{-27}(m/M_\oplus)\ s^{-3}$, where $M_\oplus$ is the mass of the Earth.

Comparing the above values with those in Table 1 we see that the orbital motion of a pulsar caused by a distant planet of a small mass may give large values of $\ddot{\nu}$ but a negligible change in $\dot{\nu}$ (in comparison with typical slowing down rates of pulsars). This shows that a large value of $\ddot{\nu}$ hints that the pulsar may be accompanied by a distant planet and therefore should be closely watched in the future. Unfortunately, the extensive timing project at Arecibo[1] was discontinued in 1976.

Figure 1 shows the residuals $N_{\rm obs} - N(t_{\rm obs})$ for PSR0329+54 with $N(t)$ given by equation (2). There is an apparent periodic component which shows up when we use data covering any period longer than five years. Let us assume that

$$N(t) = N_0 + \nu t + \tfrac{1}{2}\dot{\nu}t^2 + \tfrac{1}{6}\ddot{\nu}t^3 + a \sin\frac{2\pi}{P_{\rm orb}}(t - t_0) \qquad (3)$$

Using the least squares procedure one gets $N_0 = -0.352937 \pm 0.000296$, $\nu = 1.3995435677615 \pm 1.32\times10^{-11}$ s^{-1}, $\dot{\nu} = -4.021220\times10^{-15} \pm 2.56\times10^{-19}\ s^{-2}$, $\ddot{\nu} = 5.553\times10^{-26} \pm 2.05\times10^{-27}\ s^{-3}$, $a = (1.201\pm0.142)\times10^{-3}$, $P_{\rm orb} = 1{,}105.2 \pm 25.2$ d and $t_0 = 518.3 \pm 29.7$ d. All these values refer to the epoch JD 2,440,621.5. The errors assigned to these values are twice the formal standard deviation as derived from the least square fitting process with measurement errors assumed to be 500 μs (ref. 5); $\chi^2 = 315$, the number of degrees of freedom of the fit is $252 - 7 = 245$ and the r.m.s. residual between the fit and the data is 559 μs.

The sinusoidal term corresponds to a displacement with an amplitude of only 257 km over the period of 3 yr! This term

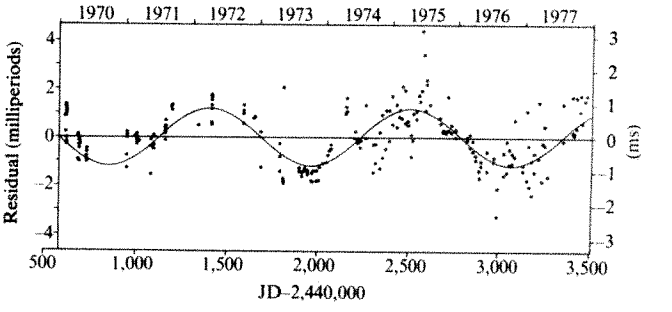


Fig. 1 The residuals for PSR0329+54 after subtraction of a best fitted cubic polynomial. The r.m.s. residual between the fit and the data is 774 μs; $\chi^2 = 603$ for $252 - 4 = 248$ degrees of freedom. The best fitting sinusoid (see equation (3) of text) is also marked.

could result either from a periodic displacement of the emission region in the pulsar magnetosphere caused by precession of the magnetic axis or a very small (858 μs) modulation of the pulsar profile which might have passed unnoticed. A small planet around the pulsar could cause the same effect. The mass function derived from the fitted parameters is $5.57 \times 10^{-19} M_{\odot}$. Masses of neutron stars are believed to be in the range of 0.1–$3 M_{\odot}$ so the lower limits on the mass of a companion ($\sin i = 1$) are correspondingly $0.06\ M_{\oplus}$ and $0.57\ M_{\oplus}$.

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VLBI Observations of the double QSO, 0957+561 A, B

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Walsh *et al.*[1] originally suggested that the double QSO 0957+561 A, B might be images of the same object formed by a gravitational lens because of their remarkably similar optical spectra. Additional optical spectral data[2] strengthen the evidence for a gravitational lens. Radio data at 5 GHz have shown a complex structure[3,4]. There are compact (<1 arc s) components closely coincident with the two QSOs; Pooley *et al.*[3] suggested that the one near B may be extended ~1.5 arc s, but the higher resolution data of Roberts *et al.*[4] make it probable that B is, in fact, also compact. In addition, there are at least two extended components, one to the north-east producing more than half the total 5-GHz flux density, and a much weaker one to the west. Pooley *et al.* show that these two extended components have fairly steep spectral indices (−0.65) between 5 GHz and 408 MHz but the components close to the QSOs have relatively flat spectra. We have now tested the gravitational lens hypothesis using the greater resolution obtained by very long baseline interferometer (VLBI) measurements of the QSOs, with the European Network at 1,666 MHz. We have detected two apparently unresolved (≤20 marc s) sources with about the same separation as the optical objects and with flux densities of 44 and 32 mJy. These sources have the same flux density ratio as the small-diameter components detected in lower resolution 5-GHz maps but we see no evidence for the extended features in these maps. Our high resolution results are consistent with the gravitational lens hypothesis.

Our observations of 0957+561 were made at 1,666 MHz using the Max-Planck-Institüt für Radioastronomie (MPIfR) 100-m telescope at Effelsberg, FRG, the Netherlands Foundation for Radio Astronomy 25-m telescope at Dwingeloo, the Netherlands, and the 76-m Mark IA telescope at Jodrell Bank, UK. The data were recorded with a 2-MHz bandwidth using the Mark II VLBI system[5] and processed with the MPIfR VLBI processor in Bonn. A hydrogen maser frequency standard was used at Effelsberg and rubidium standards were used at the other sites. A total of 5 h of observations was obtained during the period 2–4 June 1979. Table 1 summarises the observation periods. Observations of the relatively strong sources BL Lac and 0Q208 showed that the stability of the ionosphere and the local oscillators was sufficient to allow coherent integrations of up to 20 min with only ~5% loss of amplitude.

The longest baseline, Effelsberg–Jodrell, gives lobe spacings of ~55 marc s, almost independent of hour angle during these observations. It is also the most sensitive, with r.m.s. noise of ~6 mJy in a single 20-min coherent integration. With this integration time, the observations of 0957+561 show two well-resolved peaks in the fringe-frequency spectrum. Their separation varies with interferometer hour angle in a way consistent with the separation of the two optical QSOs, A and B. The fringe rate spectrum obtained with a 20-min integration centred at UT 1750, 2 June, is shown in Fig. 1. There is no significant variation in the flux density of A or B with hour angle on 4 June, the individual flux densities being 38 ± 5 mJy and 30 ± 5 mJy, respectively. Note that these errors are a quadratic sum of random noise and an estimated systematic calibration error of 12%. On the Effelsberg–Dwingeloo baseline we have approximately three times less resolution and observations are ~30% less sensitive. Again, there seems to be no variation in the component flux densities with hour angle, the mean values of A and B being 44 ± 6 mJy and 30 ± 6 mJy. From all the 4 June observations we conclude that both components are unresolved. However, for the single half-hour observation on 2 June the flux densities of A and B measured on the Effelsberg–Jodrell baseline are 62 ± 9 mJy and 43 ± 9 mJy. This might be taken as evidence for resolution, variability or even circular polarisation of the components, but we consider that the apparently greater flux densities measured on 2 June can be ascribed to calibration uncertainties. Our best estimates of the flux densities of A and B are therefore the weighted means of these various values; thus, $A = 44 \pm 4$ mJy and $B = 32 \pm 4$ mJy. Some support for the view that the components are unresolved in all the observations comes from the fact that the ratios of flux densities A/B are never significantly different from each other, the mean ratio being 1.38 ± 0.08. The sensitivity of the Dwingeloo–Jodrell baseline is one-third that of Effelsberg–Jodrell, and although we detect the source, we are unable to deduce anything further about its structure.

There is no evidence of any other radio component from our VLBI observations; from the Effelsberg–Jodrell baseline we put an upper limit of 20 mJy on any compact structure associated with the NE and W radio components reported by Pooley *et al.*[3].

It is not possible to obtain useful absolute positions for components A and B from the individual fringe rates. However,

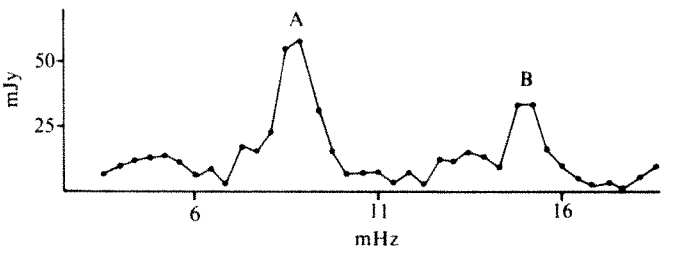


Fig. 1 Residual fringe rate spectrum of 0957+561 A, B after 20 min integration. Effelsberg–Jodrell baseline, 17.50 UT, 2 June.

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Table 1 List of observation periods

Date	UT	Polarisation
2 June	17.30–18.00	Right hand circular
4 June	08.40–13.00	Left hand circular

we can determine a very precise relative position from the relative phases by using the fringe rate differences to determine the unknown number of integral turns between the phases of A and B. The relative position and standard deviation, in the sense A minus B, from a least squares fit to the relative phases on both baselines, is $\Delta\alpha(1950.0) = -0.1453 \pm 0.0003$ s, and $\Delta\delta(1950.0) = +6.047 \pm 0.002$ arc s. The separation is 6.175 arc s. These values agree well with the 5-GHz results of Pooley *et al.*—$\Delta\alpha = -0.16$ s, $\Delta\delta = 6.1$ arc s (1950.0), separation 6.25 arc s—but are somewhat greater than the optical separation (5.7 arc s) given by Walsh *et al.* Further optical observations are needed to determine whether this difference is real.

In comparing our VLBI results with previous observations, we note first that the 5-GHz flux densities of Pooley *et al.* for A and B (48 and 37 mJy, respectively, flux ratio, A/B, = 1.30) are somewhat higher than those of Roberts *et al.* (36 and 30 mJy, respectively, flux ratio, A/B, = 1.20). These differences may be within the range of observational error. Our VLBI flux densities at 1,666 MHz (44 and 32 mJy, respectively, flux ratio, A/B, = 1.38) are very similar to the 5-GHz values. At first sight the apparent similarity of the spectral indices is further evidence for the hypothesis that we are seeing two images of the same object via a gravitational lens. However, this apparent similarity must be treated with caution for at least two reasons. (1) The 5-GHz observations refer to angular scales of the order of 1 arc s, whereas the VLBI observations refer only to compact components two orders of magnitude smaller, and we do not know what fraction of the 5-GHz flux is contained in these compact components; (2) if a gravitational lens is operating, and if, as suggested by Pooley *et al.*, the source is radio variable, then, taking into account the relative delay between the two paths, we do not necessarily expect the same spectral index for the two images.

The most important feature of our VLBI data is that we find two unresolved radio components with comparable flux density at 1,666 MHz close to the optical QSOs. As concluded by Pooley *et al.*, this is consistent with the gravitational lens hypothesis but because of the much tighter limits on angular size it is a somewhat more stringent test.

If the radio components are, in fact, images of a single object formed by a lens, our failure to resolve them allows an estimate of an upper limit to the size of the object. Adopting the notation and formulae quoted by Pooley *et al.*, the maximum linear magnifications of the two images (transverse to the direction of the line joining them) are α_1/β and α_2/β, respectively. The values of α_1, α_2 and β can be determined from the flux ratio, which is equal to α_1^2/α_2^2. Unfortunately, the frequency-independent flux ratio inherent in the geometrical optics of the lens is uncertain because of variability, which, as discussed by Pooley *et al.*, seems to be necessary on lens theory. The closer the flux ratio is to unity, the greater the magnification; thus, a reasonable estimate of the upper limit to the intrinsic size of the radio object may be obtained by adopting the largest value of the flux ratio yet proposed. This is the value derived from the optical spectrum by Carswell and D.W. (in preparation), who suggest that after correction for dust close to the QSO, the flux ratio is 1.5 (in the sense B/A). This then yields $\alpha_1 = 3.40$ arc s, $\alpha_2 = 2.78$ arc s, $\beta = 0.62$ arc s and maximum linear magnifications of 5.5 and 4.5 for B and A, respectively. This implies an upper limit of ~4 marc s for the equivalent gaussian diameter of the radio object, and an upper limit to the flux density of ~15 mJy if the lens were absent. Note that the formulae from which this result is obtained are based on the assumption of a point deflecting mass. For the more realistic case of the distributed mass of a galaxy, the precise formulae will depend on the mass distribution, but as long as the flux ratio is not greatly different from unity, the above estimates are probably reasonable.

An upper limit of 20 or even 4 marc s to the diameter of a radio component is quite consistent with that expected for a weak synchrotron source in the nucleus of a QSO, so our size measurements do not provide a definitive test of the lens hypothesis. However, VLBI observations with at least an order of magnitude greater resolution are feasible, and it might then be possible to determine the structures of the two components and so provide a much stronger test of the lens hypothesis.

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Galactic radiation belts

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Several decades of observations have failed to resolve the problem of the interpretation of extended extragalactic radio sources[1,2]. Most current models invoke the ejection of pairs of plasmoids or relativistic electron beams from the parent elliptical galaxy by various mechanisms[3–5]. As these theories generally predict the relative orientations of the rotation, magnetic and radio source axes, the measurement of position angles provides one test of the proposed models[6,7]. However, position angle studies[6–11] have produced ambiguous and sample-dependent results. While recent analyses[6,7] indicate a preference for radio source alignment along the inferred rotation axes of the optical counterparts, other investigations found random distributions of position angle or bimodal distributions with peaks separated by ~90° (refs 8, 10). One alternative interpretation of extragalactic radio sources that has received little previous consideration, and is considered herein, concerns the possibility that the emission arises from belts of trapped electrons encircling the parent galaxy in the same manner as the Van Allen belts encircle the Earth. This idea seems to have been dismissed primarily because of the absence of ring-shaped or toroidal emission patterns in the radio observations[12]. The morphology of synchrotron emission patterns from these hypothetical structures, however, has never been investigated in detail.

An integral describing the two-dimensional brightness distribution on the plane of the sky from synchrotron-emitting relativistic electrons trapped in a dipolar magnetic-field shell was formulated by Ortwein *et al.*[13] for analysis of the jovian radio emission. These authors derived their results for an arbitrary dipole-axis orientation angle θ_0 with respect to the line of sight and for various degrees of anisotropy of the trapped-electron distribution. The latter was parameterised by the loss-cone angle α_L, which is the smallest populated equatorial pitch angle in the otherwise isotropic particle distribution, and the power law energy spectrum index γ.

While the calculated emission patterns were insensitive to the value of γ, they were strongly dependent on both the aspect

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angle and the loss-cone angle. The emitting shell of electrons assumed one of six different morphologies for various combinations of θ_0 and α_L: (1) the solid ellipsoid, sometimes showing complex internal structure, (2) the annulus, (3) the limb-brightened, elongated annulus, which approached in appearance (4) the limb-brightened dumbbell shape, with source axis perpendicular to the dipole axis, (5) the separated double source, with source axis parallel to the dipole axis, and finally (6) the invisible source. Figure 1 shows some of these forms.

For the present investigation, the numerical integrations described by Ortwein *et al.*[13] were performed for a large number

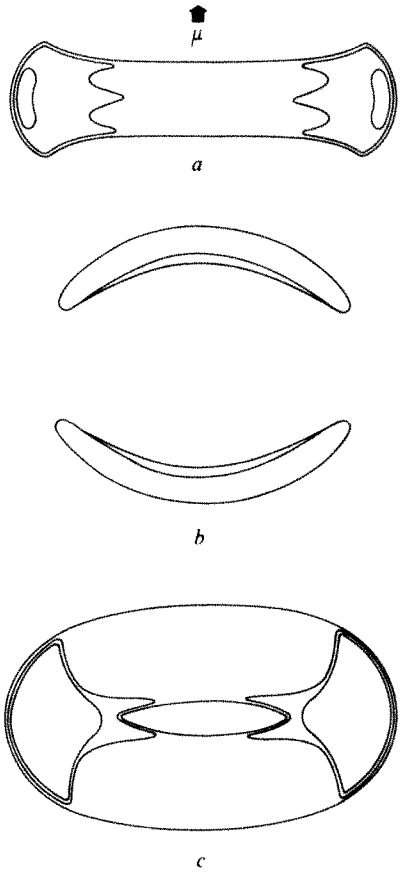


Fig. 1 Examples of the brightness distributions produced by calculations of synchrotron emission from a dipolar shell of trapped electrons using the method described by Ortwein *et al.*[13]. A dumbbell distribution (*a*) with source axis perpendicular to the dipole moment μ is produced when the aspect angle θ_0 is 90° (line of sight perpendicular to the dipole axis) and the loss cone angle is $\alpha_L = \sin^{-1} 0.8$. A double source with source axis parallel to the dipole axis (*b*) is obtained when $\theta_0 = 35°$ and $\sin \alpha_L = 0.8$. The limb-brightened, flattened annulus or torus (*c*) results from the parameters $\theta_0 = 60°$ and $\sin \alpha_L = 0.6$. The contour interval is variable. Numerical results can be found in the work of Ortwein *et al.*[13].

of aspect angles θ_0 between 0 and 90° and loss cone angles α_L between 0 and 90°. Five-degree intervals were taken in aspect angle θ_0 and $\sin \alpha_L$ was incremented by 0.05. The electron spectral index γ was set equal to the nominal value of 1.5, which is the galactic cosmic-ray index[14]. Two-dimensional plots of the intensity were generated for each case. These were then categorised according to morphological class as defined by the six types described above.

Figure 2 shows how the morphology of the brightness pattern varies as a function of aspect angle and electron pitch angle anisotropy. In nature, the dipole axes will be isotropically distributed. However, all loss-cone angles may not occur. In particular, the results given in Fig. 2 indicate that if large loss cones ($\sin \alpha_L > 0.8$) are prevalent, the observed distribution of

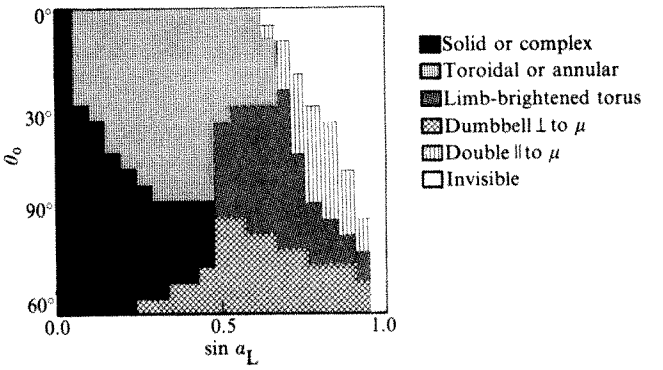


Fig. 2 Distribution of source morphologies for all values of aspect angles θ_0 and loss-cone angle α_L.

source morphologies will be practically equivalent to what has been found in some of the data analyses. These thin disk-like populations of gyrating electrons produce emission patterns that are virtually undetectable or double with the radio source axis either parallel or perpendicular to the dipole axis of the parent galaxy. (The limb-brightened annuli are likely to appear as doubles connected by 'bridges' of emission.)

Of course, actual galactic radiation belts would be composed of a distribution of non-uniformly emitting shells, thus altering the emission patterns somewhat. For example, it would not be surprising to find (under circumstances of non-uniform sources, sinks and field geometry) cases of multiple belts which appear as nearly co-linear paired sources. Also, galaxies with minimum-*B*

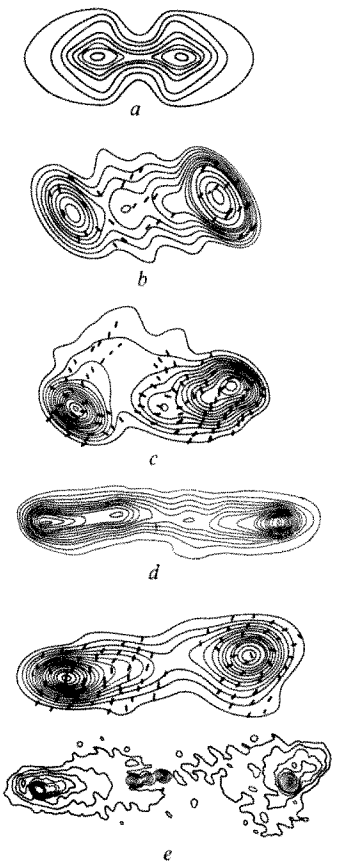


Fig. 3 A selection of observed radio-source brightness distributions at various resolutions: *a*, Jupiter[17] at 10.4-cm wavelength; *b*, 3C234 (ref. 16) at 1.4 GHz (21 cm); *c*, 3C382 (ref. 16) at 1.4 GHz; *d*, 3C452 (ref. 1) at 1.4 GHz; *e*, 3C219 (ref. 16) at 1.4 GHz (top) and at 2.7 GHz (bottom). The dashes show the direction of the magnetic field in the sources as inferred from polarisation measurements.

surfaces that are warped by non-dipolar field components[15] may show an anomalous displacement between the rotation and magnetic axes, as well as asymmetric trapping regions. Moreover, a galactic wind[24] or local intergalactic material can further distort the configuration of trapped electrons. Figure 3, which compares the brightness distributions and inferred magnetic field geometries of several extragalactic radio sources[16] with the jovian radiation-belt source[17], suggests an intriguing similarity.

The related questions of why galactic radiation belts should exist at all and why they would be populated by electrons with such a highly anisotropic pitch angle distribution lead to several speculations. Parker[18] demonstrated that the magnetic field of our own galaxy could be attributed to dynamo activity. Dipolar components are preferentially excited in dynamos and dominate the field at large distances from the dynamo centre[19]. Also galaxies contain sources of energetic particles, the cosmic rays. Various scattering mechanisms and propagation effects[20,21] can cause particles originating in the galaxy to become trapped in a surrounding dipolar field which is effectively a highly organised cosmic-ray halo. In fact, the cosmic ray spectrum observed in Earth's vicinity could produce the observed radio source spectra[22]. Finally, Schulz[23] pointed out that both synchrotron losses and radial diffusion processes in radiation belt configurations naturally lead to pitch angle distributions that are strongly peaked at an equatorial pitch angle of 90°.

Many peripheral points of interest can be found in analogies with other radiation belts in nature[23,25]. The perpendicular sources may show evidence of the gradient-curvature drift of the trapped particles around the parent galaxy. This azimuthal drift would cause opposite Doppler shifts of the synchrotron radiation from the two extremities of the source. Magnetic storms, analogous to those which accelerate particles in Earth's environment[26], may have their counterparts in galactic magnetospheres. The variation of the radio sources with time will be determined by the competition between particle sources and synchrotron losses, and also by changes in magnetic field geometry. Some 'tailed' radio sources have already been compared with the Earth's magnetosphere[27,28].

These speculations notwithstanding, the calculations described above suggest that electrons trapped in a dipolar magnetic field can reproduce some of the observed distributions of emission from extended extragalactic radio sources, including the absence of toroidal configurations, if the electron pitch angle distributions are sufficiently anisotropic.

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An empirical determination of the heating of the Earth by the carbon dioxide greenhouse effect

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Numerous theoretical calculations[1,2] have been made of the effect of an increased carbon dioxide concentration in the atmosphere on the surface temperature of the Earth. Estimates of the increase in the surface temperature of the Earth caused by a doubling of the carbon dioxide content of the atmosphere generally range from ~0.7 to 2.9 °C. For a 10% increase in carbon dioxide, the corresponding temperature increases range from 0.096 to 0.40 °C. All these results are calculated from theoretical models which range in complexity from one-dimensional planetary radiation budget studies to three-dimensional general circulation models. Here a new approach to this problem is described which allows an empirical determination of the heating of the surface of the Earth by the carbon dioxide greenhouse effect to be calculated. This technique indicates that the heating of the Earth over the period 1880–1970 was 0.40 °C or less.

Consider that climatic fluctuations have three components: (1) natural climatic changes caused by external forcing such as variations in solar luminosity; (2) natural climatic fluctuations caused by year-to-year stochastic variations in sea-surface temperature, snow and ice cover, cloud cover, and so forth; and (3) man-induced climatic variations caused predominantly by increased carbon dioxide in the atmosphere. Here most of the observed trends in the Earth surface temperature over the last century are hypothesised to have been caused by external forcing, namely by changes in solar luminosity. Once we know the history of this forcing function, the forcing of climate caused by the carbon dioxide greenhouse effect can be determined. If changes in volcanic activity contribute significantly to long-term trends in climate, then the estimate for the carbon dioxide warming will need to be altered. The results of an earlier study[3] are now reviewed so that we can reconstruct the solar luminosity variations over the last century.

Sunspots are dark regions on the Sun which vary in number with an 11-year cycle. Nearly all sunspots have a dark central region (umbra) and a less dark surrounding region (penumbra). The areas of these regions have been measured by the Royal Greenwich Observatory since 1874[4]. The ratio of the areas of the umbrae to that of the penumbrae may be used to characterise sunspot structure. The umbral/penumbral ratio varies not only from sunspot to sunspot but also has marked year-to-year variations and shows long-term secular changes (Fig. 1).

The umbral/penumbral ratio may be a measure of the amount of convective energy transport in the photosphere of the Sun[3,5–7]. Facular activity is more intense when the umbral/penumbral ratio is high[5], indicating that the umbral/penumbral ratio is directly proportional to the solar convective flux. Deszo[8] indicates that the manner in which sunspots decay in time also implies that the umbral/penumbral ratio is a measure of solar convective flux. For periods of more active convective motions, the penumbrae tend to be destroyed more readily than the umbrae hence increasing the umbral/penumbral ratio.

If the umbral/penumbral ratio is a measure of convective flux in the Sun, it must also be a measure of radiant flux from the Sun. During periods of more intense convective flux, warmer gases from inside the Sun will rise to the surface and the Sun will be brighter. An earlier study[3], using the mixing length theory of convection and available sunspot models, showed that the observed changes in the umbral/penumbral ratio were

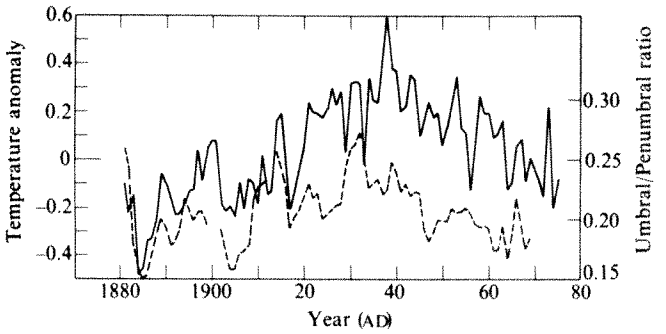


Fig. 1 The solid curve shows the Northern Hemisphere temperature anomalies from Budyko (1881–1957), Reitan (1958–1968) and Angell and Korshover (1969–1975). The dashed curve illustrates the umbral/penumbral ratio from the Royal Greenwich Observatory measurements 1881–1969. Note the general similarity, particularly for the earlier years.

equivalent to a change on the solar constant of about 0.4% over the last century.

A consequence of the above is that the climate of the Earth will vary in a parallel manner with variations of the umbral/penumbral ratio. The higher the umbral/penumbral ratio, the greater will be the solar constant and the warmer the Earth. Figure 1 compares the temperature anomalies of the Northern Hemisphere[9-11] and the umbral/penumbral ratio. The cross-correlation of these two time series is 0.53 ± 0.13 which is significant at better than the 1% level. The uncertainty of this correlation has been increased[12] to correct for the autocorrelation in the two time series. The umbral/penumbral time series has been corrected for the years 1900, 1901 and 1913 as suggested earlier[3], although using years with few sunspots causes the correlation to be less than it would be otherwise. Using only those years for which the penumbra covers more than 100 millionths of the solar disk gives a cross-correlation of 0.57. The probability of such a high cross-correlation occurring by chance is < 1 in 10^2. Furthermore, when both series are detrended by fitting parabolas to each and subtracting them from the observed time series, the cross-correlation of the residuals equals 0.46. It is the common year-to-year variations in the two time series that gives rise to their correlation rather than their common long-term trends.

Although the umbral/penumbral ratio successfully accounts for ~28% of the total variance in the observed temperature records, the crucial test of this mechanism for climatic change is that solar constant variations be proportional to the umbral/penumbral ratio. The predictive capability of the model as it stands supports its validity. In particular, it has successfully predicted the following: (1) the umbral/penumbral ratio should be small during the Maunder Minimum of the late 1600s (ref. 13). A preliminary qualitative check of available sunspot drawings agrees with this prediction[3]. (2) Because of the need for energy balance in the solar convective zone, the solar rotation rate should have increased in recent years: Measurements by Livingston and Duvall[14] confirm this prediction. (3) Correspondingly the solar rotation rate before 1930 when the Earth was warming should have decreased to a minimum about this time: measurements by Eddy[15] apparently confirm this prediction. (4) Because of a 20-yr cycle in the umbral/penumbral ratio[3], there should be a 20–22-yr cycle in certain meteorological parameters. The best documented such variation concerns the 22-yr cycle of droughts in the western US[16]. The umbral/penumbral ratio has peaked 0–4 yr before each drought over the past century[3] and strongly suggests a physical connection. (5) The thermal time constant of the Earth–ocean–atmosphere system should be ~4–5 yr based on calculations using a simple energy balance climate model. Others[17-18] have estimated this time constant to be ~6.5–6.9 yr. Further information on this hypothesis for climatic change is given elsewhere[3].

The proposed hypothesis should also allow a successful prediction of the warming of the Earth by the carbon dioxide greenhouse effect. Only three basic assumptions are needed (1) the umbral/penumbral ratio and surface temperature of the Earth are correlated and proportional to one another. Although for physical reasons the umbral/penumbral ratio is believed to provide a measure of the solar constant whose variations in turn cause climatic change, another physical mechanism could be invoked without upsetting the above assumption. The record for the surface temperature of the Northern Hemisphere is taken to be representative of the whole Earth[19] although doubts have been expressed about the parallelism of the temperature records of the two hemispheres[20]. (2) The carbon dioxide content of the atmosphere has been increasing exponentially since 1880 (refs 21, 22) and consequently the temperature of the Earth has been increasing approximately exponentially with time[1]. (3) Earth surface temperatures have long-term secular variations over the past 100 yr forced predominantly by variations in solar luminosity and carbon dioxide increases. Long-term trends in atmospheric transmission attributable to anthropogenic or volcanic aerosols reported by Budyko[9] as a possible cause of climatic change have not been supported by many subsequent studies[29-33]. Volcanic activity or natural variations may contribute to the trend in temperature but for the first estimate of the carbon dioxide warming these effects are neglected.

The trends in the observed temperature record[9-11] (Fig. 1) with the above assumptions consist of a natural variation caused largely by solar variations and anthropogenic portion caused by increased carbon dioxide. The anthropogenic portion is analytically represented in two models by a linear and exponential increase of temperature with time. Subtracting various hypothetical anthropogenic temperature increases from the observed temperature records allows natural temperature variations to be deduced. The highest cross-correlation of a calculated natural temperature variation versus the umbral/penumbral ratio is 0.63 and occurs for an assumed linear anthropogenic temperature increase of 0.40 °C over the period 1880–1970 (Fig. 2) or assumed exponential increase of 0.3 °C to 0.4 °C. The deduced increase in temperature caused by anthropogenic carbon dioxide should be interpreted as an upper limit to the actual warming because the presumed cooling in the late 1880s caused by volcanic eruptions[23] will tend to be interpreted as a natural climatic variation. Hence, the warming after the volcanic eruptions will give a higher value for the calculated carbon dioxide warming than it should. A more detailed climate model should help resolve the magnitude of this overestimation. The location of temperature measurements near urban areas may also give the appearance of a long-term warming of the Earth because of the increasing effects of urban heat islands[19]. The observed and expected natural temperature variations in the absence of mankind are shown in Fig. 3.

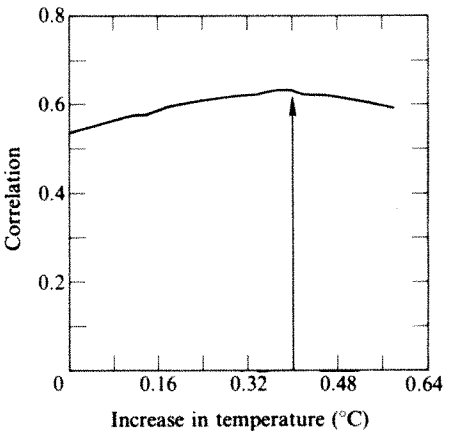


Fig. 2 The correlation of the natural temperature anomalies compared with the umbral/penumbral ratio for various assumed linear temperature increases caused by the carbon dioxide greenhouse effect during 1880–1970. The best correlation occurs for a linear temperature rise of 0.4 °C. A similar plot for a more realistic exponential temperature rise gives a best correlation for 0.3–0.4 °C.

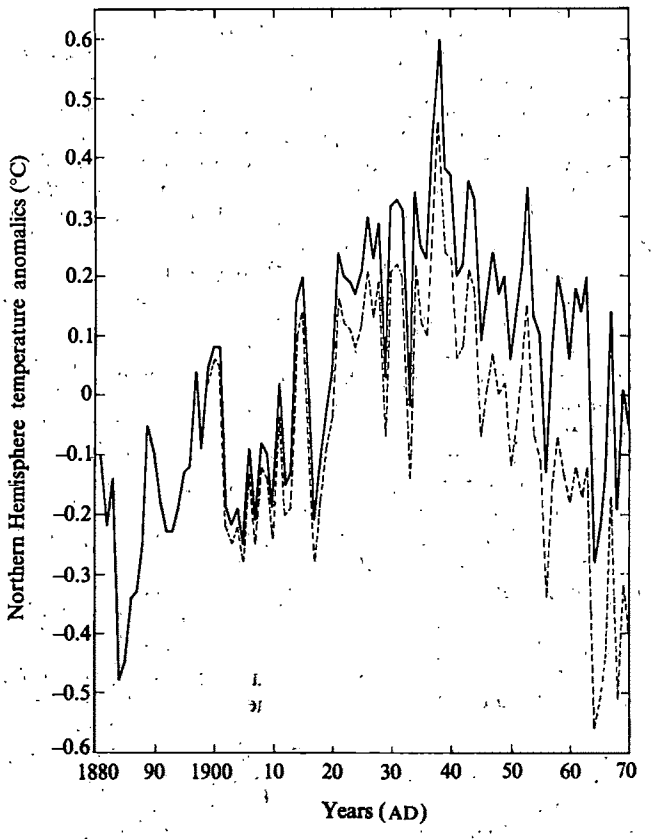


Fig. 3 The solid curve gives the observed Northern Hemisphere temperature anomalies and the dashed curve gives the natural unperturbed Northern Hemisphere temperature anomalies. The increasing exponential separation of the two curves is caused by the increased (anthropogenically produced) carbon dioxide.

Although the 0.30–0.40 °C warming due to the carbon dioxide greenhouse effect is the present best estimate of this quantity based on techniques used here, there is considerable uncertainty in the statistical significance of this result. The cross-correlation between the calculated externally driven natural temperature variation and the umbral/penumbral ratio is 0.63 ± 0.13. The cross-correlation between the observed temperature variation and ratio is 0.53 ± 0.13. As only 90 yr of data are used in calculating these correlations, the significance of the difference between the correlations is only 16% (ref. 24). Although this technique does not prove that the Earth is warmer because of the presence of added carbon dioxide in the atmosphere, it supports this hypothesis.

Estimates of the carbon dioxide content in the atmosphere in 1880 range from 280 (ref. 21) to 293 p.p.m. (refs 23, 25). If during 1970 the concentration was 320 p.p.m. (ref. 26) the total increase in carbon dioxide from 1880 to 1970 was 9–14% of the original amount. The 0.40 °C temperature increase therefore implies that doubling the amount of carbon dioxide would result in warming in the range 2.0–3.1 °C. Manabe and Wetherald[27,28] estimate a 2.4 and 2.9 °C temperature increase respectively for the same conditions. The Manabe and Wetherald models imply that for a 9–14% increase in carbon dioxide, the Earth's surface temperature increases by 0.30–0.55 °C which bracket the present 0.3–0.4 °C empirical prediction. The present results further predict that a continued exponential increase in carbon dioxide content of the atmosphere, will warm the Earth by an additional ~0.3–0.4 °C by the year 2000.

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Evidence for quasi-periodic July drought in the Hudson Valley, New York

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July Palmer Drought Severity Indices[1] (PDSI) have been reconstructed[2] for the Hudson Valley region of New York State from the annual ring-width variations of local old trees. Using additional, subsequently developed tree-ring chronologies, we have developed a new July drought reconstruction that extends back to 1694. Variance spectra of the new PDSI series reveal statistically significant quasi-periodicities of 11.4 and 26 yr.

The July PDSI reconstruction was developed using six tree-ring chronologies from sites in the Hudson Valley. Each chronology is represented by only one tree species and is a mean-value function of 30–40 ring-width series from individual trees on a site. Five tree species are represented in the six chronologies: eastern hemlock (*Tsuga candensis* Carr.), pitch pine (*Pinus rigida* Mill.), eastern white pine (*P. strobus* L), chestnut oak (*Quercus prinus* L.) and white oak (*Q. alba* L.). Using several species and sites practically eliminates the possibility that insect infestations or other non-climatic disturbances have created the fluctuations found in the drought reconstruction. The reconstruction was developed using a combination of principal components analysis and multiple regression to compute prediction equations for estimating past climate[3,4].

Figure 1 shows the tree-ring PDSI estimates (*a*) plotted against the actual Hudson Valley regional July PDSIs (*b*). The latter series was used as the dependent variable to develop the multiple regression calibration equation for reconstructing drought. The estimates reduce 54% of the variance in Fig. 1*b* adjusted for degrees of freedom[5]. This is statistically significant well below the 1% level. The July PDSIs in Fig. 1*c* were computed from monthly temperature and precipitation data of the Mohonk Lake, New York cooperative meteorological substation which is near some of the tree-ring sites. The 1896–1930 interval is independent of the calibration period and, thus, can be used to test and verify the accuracy of the regression estimates. Three tests of verification were used: the sign test, the product means test[3] and the degree of cross-correlation. The sign test (30 agreements, 5 disagreements) and the correlation coefficient ($r = 0.60$; d.f. = 33) pass the 1% significance level. The product means test ($t = 2.21$; d.f. = 33) passes the 2.5% significance level.

To test the frequency-domain fidelity of the reconstruction, we computed spectral and cross-spectral estimates[6] of the

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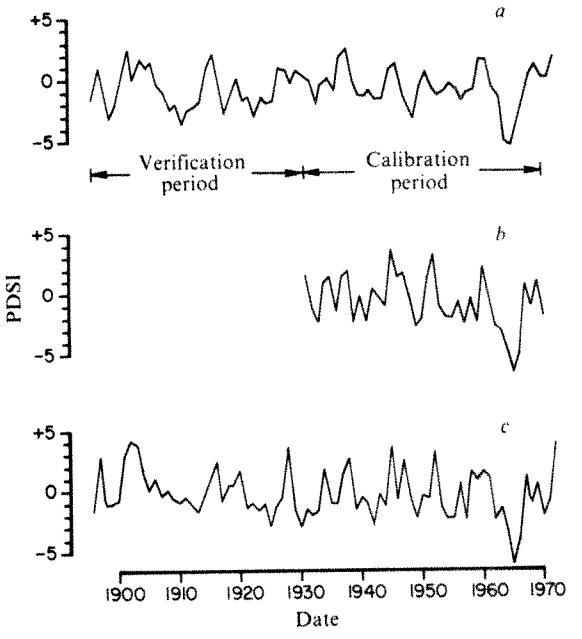


Fig. 1 Tree-ring reconstructed July PDSI (*a*), Hudson Valley Division July PDSI (*b*) used to calibrate the multiple regression equation for estimating PDSI from tree growth, and Mohonk Lake, New York. July PDSI (*c*) used to verify the tree-ring PDSI estimates.

Mohonk Lake and reconstructed PDSI series. For this test we used all of the common data (1896–1972) to provide better low-frequency resolution. The two series are no longer independent because the Mohonk Lake data are highly correlated with the divisional data used in the 1931–70 calibration period. But as the 1896–1972 common interval is almost twice the calibration period, we still expect a reasonably rigorous depiction of the frequency-domain fidelity of the PDSI reconstruction.

Figure 2 shows the variance spectra (*a*) and the squared coherency spectrum (*b*) of the two series. The similarity of the variance spectra illustrates the good fidelity of the drought signal recorded by tree rings. The loss of variance in the higher frequencies of the reconstruction is partly a function of the unexplained variance of the regression equation used for prediction. Note that both spectra have a broad-based peak centred at a period of 12.6 yr.

The coherency spectrum provides an estimate of the common per cent variance between two series at each frequency. This spectrum indicates that 60–80% of the climatic signal in the bandwidth accounting for periods of 7–30 yr is common to both series. Other frequencies show poor to good coherence. The phase spectrum is not shown because the two series are in phase for almost all frequencies.

Figure 3 shows the complete July PDSI reconstruction and a low-pass filtered version that passes all variance at frequencies of once in 10 yr or less. Judging from the coherency spectrum, the filtered PDSI series shows the most reliable drought information. It clearly shows a strong low-frequency rhythm of wetness and dryness that is suggested in Fig. 2.

Using the 1700–1972 period of the unfiltered series, we computed a high-resolution variance spectrum from the autocovariance function of $N/3$ or 91 lags. The Hamming window was used for smoothing the spectrum. Figure 4*a* shows two very strong peaks of variance at periods of 11.4 and 26 yr. The general shape of the spectrum is characteristic of a time series containing 'red noise' or persistence which inflates the low-frequency variance. Significance tests of such spectra must be computed either from a null spectrum of an empirically determined low-order Markov process[7] or after prewhitening to reduce the original data to residual 'white noise'. We use both approaches with an *a priori* hypothesis test, but compute the confidence levels from somewhat different null models.

The null spectrum of the unwhitened PDSI data was computed from the autocovariance function of 14 lags which produces a very low resolution spectrum. In this case, we want the null spectrum to be arbitrarily smooth without defining the stochastic process it represents. Using a one-tailed χ^2 test, the significance levels are computed as

$$\mathrm{CL} = \frac{\Gamma(\omega)\cdot\chi^2}{\nu} \qquad (1)$$

where $\Gamma(\omega)$ is the null spectrum estimate for frequency ω and χ^2 is the χ^2 value for a given confidence level (CL) with ν degrees of freedom. In Fig. 4*a*, the 95 and 97.5% confidence levels are plotted with the high and low resolution spectra. Both the 11.4- and 26-yr peaks exceed the 97.5% level of significance.

In Fig. 4*b*, the high-resolution spectrum was computed after the PDSI series was prewhitened using the difference equation of the second-order autoregressive (AR) process

$$Y_t = A_t + 0.827\,Y_{t-1} - 0.293\,Y_{t-2} \qquad (2)$$

The order of the process was selected using the Akaike final-prediction-error criterion[8] which is an unbiased estimator of the order of an AR process[9]. Although the drought series may be more complex than an AR (2) process, the cumulative periodogram of the prewhitened series did not show any systematic departure from a white noise null continuum. For white noise

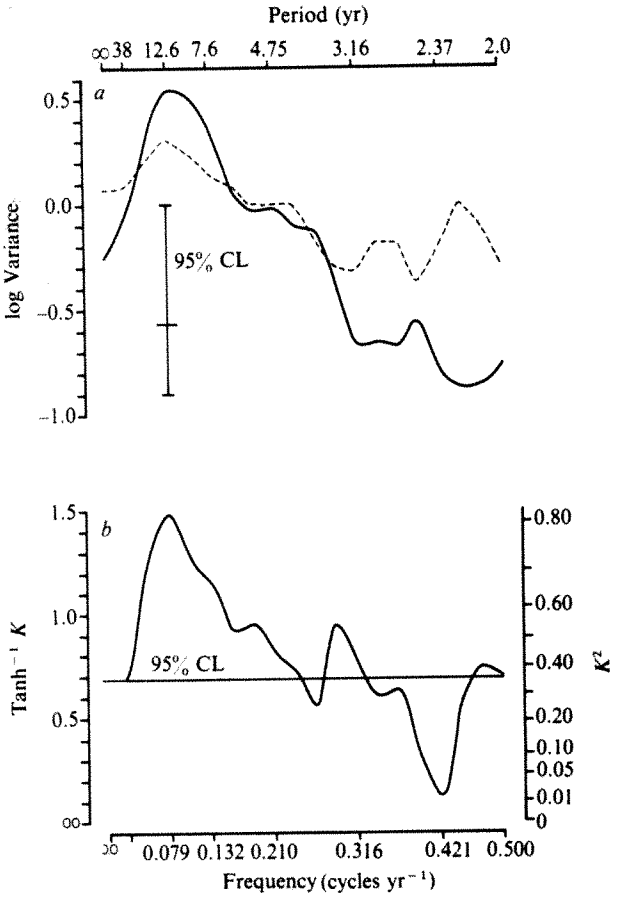


Fig. 2 Variance spectra of Mohonk Lake and tree-ring reconstructed July PDSI and the squared coherency (K^2) spectrum of the two series. Data shown in Fig. 1*a* and *c* were used to compute the spectra. The confidence interval of each variance spectrum is the same. $N = 77$; d.f. = 10; bandwidth = 0.066. Dashed line indicates Mohonk Lake, New York, July PDSI.

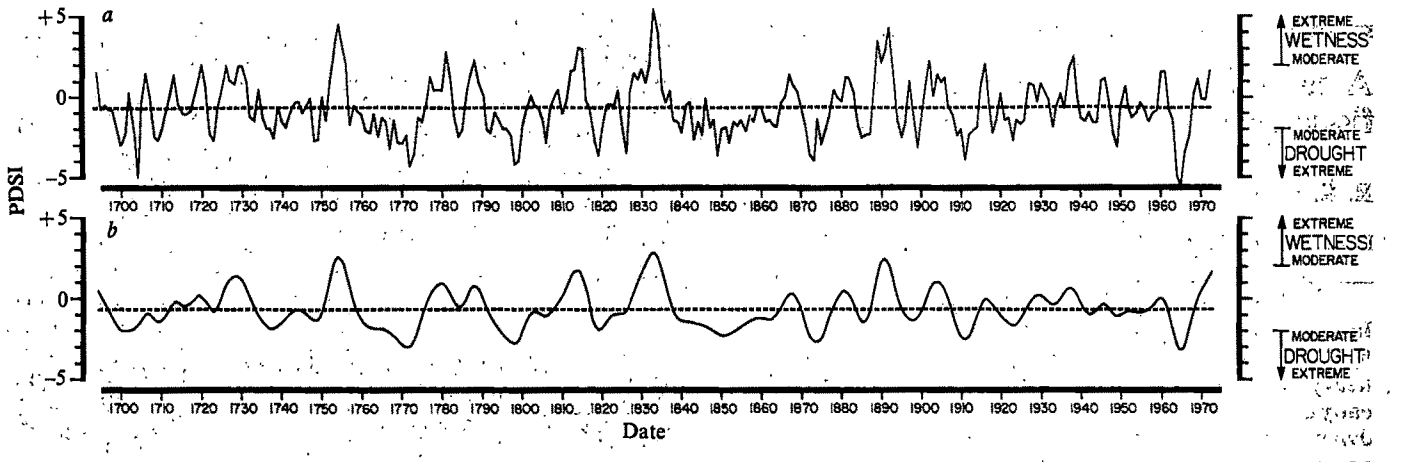


Fig. 3 Tree-ring reconstructed July PDSI from 1694 to 1972. *a*, The unsmoothed estimates. *b*, A low-pass filtered version of the unsmoothed series that emphasises frequencies of once in 10 yr or less.

processes, the expected relative variance of the null spectrum at each frequency is

$$\Gamma(\omega) = 1.0/m \qquad (3)$$

where m is the number of spectral estimates. $\Gamma(\omega) = 0.011$ for all ω in our case and the confidence levels are computed using equation (1). Again the 11.4- and 26-yr peaks are significant at the 97.5% confidence level.

We conclude that low-frequency fluctuations of past drought in the Hudson Valley are partly quasi-periodic. These periods may be identified with the 11-yr sunspot cycle[10] and a 27-yr cycle of soli-lunar tidal influence on zonal westerly flow[11]. An unsmoothed periodogram of the drought series indicates that ~20% of the variance is accounted for by these harmonics. Although this percentage suggests that these harmonics may have some practical predictive use, the nature and cause of these signals must first be understood. We strongly discourage any idea that drought in the Hudson Valley can be operationally predicted from the present results.

The belief that influences outside the Earth's atmosphere could significantly modulate climate is gaining credibility. A rigorous statistical analysis has convincingly linked the 22-yr midwestern US drought rhythm with the Hale sunspot cycle and its envelope, the Gleissberg cycle[12].

A highly coherent spatial pattern of inferred solar cycle signal (~10.7 yr) has also been detected in surface–air temperature data over North America[13]. It is particularly interesting that the presence of that signal is apparently limited to the region north of 35° N latitude and east of the Rocky Mountains divide. The Hudson Valley is within this region.

We do not yet know if there are consistent relationships between our drought series and such external forcing functions of climate. However, the 11.4- and 27-yr quasi-periodic drought rhythms are sufficiently near the soli-lunar tidal and sunspot periodicities to warrant a statistical inquiry into these possible relationships. Even if these harmonics are eventually regarded as part of an ensemble of internal stochastic mechanisms[14] of the ocean–atmosphere–cryosphere system, they represent an important aspect of climatic variability that is still poorly understood.

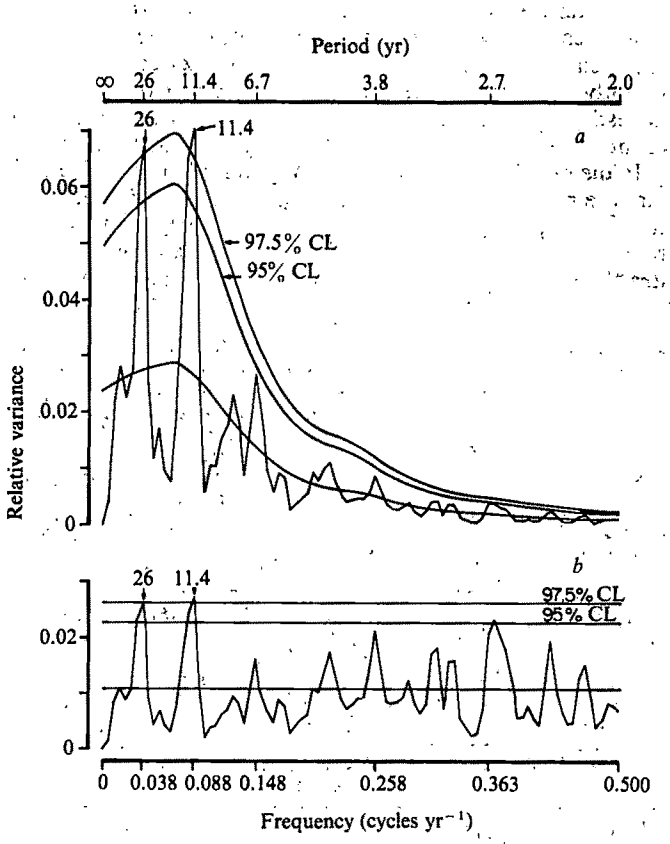


Fig. 4 Variance spectra of the unsmoothed July PDSI reconstruction (*a*) and the same series after prewhitening (*b*) using equation (2). An appropriate null continuum and its 95% and 97.5% confidence levels (CL) are superimposed on each spectrum. The bandwidth = 0.0138 and d.f. = 6 are the same in each case. $N = 273$.

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A search for superheavy-element fission-tracks in iron meteorites

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In a series of recent papers Runcorn and coworkers[1-3] have hypothesised that the radioactive decay of siderophile superheavy elements (SHE) provided the heat source which produced early melting of the Moon and which drove an ancient lunar core dynamo. If such elements had a half life of $\sim 10^8$ yr then this provides a natural explanation for the decay of the postulated lunar core dynamo and the associated magnetic field on a time scale of this order. It would be surprising if these SHE had accreted into the early Moon and not into the parent bodies of the iron meteorites. Libby *et al.*[4] have suggested that the iron meteorites did accrete SHE, and have argued that SHE fission has produced the observed peaks in the trace element abudances in iron meteorites at charge number $Z \sim 40$, 50 and 80. Libby *et al.*[4] claim that these abundances are well above those which may be expected on the basis of a simple calculation of the solubilities in Fe of these elements. These peak trace element abundances are ~10 p.p.m. by weight and assuming an element at the peak corresponds to a fission yield of 5% then the original abundance of SHE in the metal must have been in the region of 2×10^{-4} kg kg^{-1}. Such high concentrations of fissioning elements should produce a number of observable effects, one of which is the production of very large numbers of radiation damage tracks in the borders of silicate grains along the boundary with metal regions. This boundary contribution falls to zero at a distance within the silicate crystal equal to one fission fragment range (~5–10 μm). We report here a search for fissioning species in the metal phases of IA iron meteorites and a pallasite. No excesses of tracks in these boundary regions have been found and an upper limit of $\sim 10^{-12}$ kg kg^{-1} can be placed on the concentration of fissioning superheavy elements in the metal of the Odessa IA iron at the time when fission track retention began.

We have examined the silicate portions of two group IA iron meteorites and of the Brenham pallasite. In the case of the iron meteorites Odessa and Landes, slices were cut and the metal–silicate boundaries examined. A large Brenham olivine inclusion was extracted cleanly from the metallic matrix and mounted with the interface surface uppermost. One slice of Brenham was also studied.

Track densities near to the centre of each grain and along a strip ≈10 μm wide immediately adjacent to the metal contact were measured. In addition track densities on the interface surface of one olivine grain were also measured. The track densities are displayed in Table 1. Also given are the probable sources of the tracks in the central regions of the crystals.

Various criteria for deciding on the principal sources of tracks in any given meteorite crystal have been enumerated by Fleischer *et al.*[6]. In Brenham olivine, orthopyroxene from Landes and feldspar from the Odessa meteorite the tracks are observed to have anisotropic angular distributions and track length distributions dominated by short (<5 μm) tracks. These tracks would seem to result from slowed-down heavy cosmic-ray primaries. Track densities encountered in Odessa diopside are considerably higher than those in nearby feldspar crystals. The angular distribution of the tracks is isotropic and the length distribution fairly flat up to track lengths of ~12 μm (ref. 7). These characteristics all indicate a fission origin for these tracks[6] and previous work[7] has established that the majority are most probably formed by the spontaneous fission of ^{244}Pu.

These assignments of track origin are important because they determine the density of tracks to be expected in the boundary regions. For an external source such as cosmic r
track density measured in a 10-μm wide strip a
metal boundary, ρ_B should equal the central trac
For an internal source such as fission the track d
external surface of a silicate grain should be $\sim\rho_C/2$
one fission fragment range (~10 μm) within the c
~10-μm boundary strip the mean track density
measured will be approximately $0.75\rho_C$. This estir
rough indication of the expected track density si
value will depend for example on the angle made b
silicate interface to the sample surface and also o
tude of the contribution of external track sources,
it gives a rough guide for comparison with the e
results. Columns 4 and 5 of Table 1 show e
measured track densities in the boundary regio
shows a scanning electron micrograph of a typical m
boundary.

The results of Table 1 show no evidence for an exc
near to the metal–silicate boundary, except for an a
very shallow pits on the interface surface of the Bren
grain which were not visible on internal surfaces. T
have the appearance of fission tracks under the
scanning electron microscopes, being <1 μm deep
plausible explanation for their appearance is th
spallation recoil tracks resulting from cosmic ray
with heavy elements, such as Ni, in the metal phase

The lack of excess tracks in Brenham suggests an
on the SHE track density of the same order as the e
track density measurement, $\sim 2 \times 10^3$ cm^{-2}. Lande
roxene and Odessa feldspar and diopside allow
restrictive limits to be placed on the SHE track den
bution because of their higher central track densitie

It is difficult to interpret these track densities in ter
on the SHE concentrations unless the cooling histo
meteorites are known. For example, if Brenham was
a late heating event then pre-existing fission trac
removed. If the time of track retention begar
recently then most of the SHE would have decay
has estimated a half life of $\sim 10^8$ yr for the propose
elements on the basis of the decay of the lunar p

In the case of Odessa diopside, however, it ha
that the majority of the tracks are due to fissio
track density ratio $\rho_{Pu}/\rho_U = 106 \pm 9$. The age in
ratio depends on the initial Pu/U ratio. In mete
ites Kirsten *et al.*[8] have found Pu/U ratios at

Table 1 Measured and expected boundary trac

Meteorite [Class]	Mineral [no. of crystals examined]	Central track density‖ (cm^{-2}) [probable source]	Expe bou sity tr_m de
Brenham [pallasite]	Olivine* [2]	$(1.0 \pm 0.2) \times 10^4$ [cosmic rays]	(1 (deep pits ~10^4 shallow pits ~2×10^5
Landes [IA iron]	Ortho-pyroxene† [2]	$(1.50 \pm 0.18) \times 10^7$ [cosmic rays]	$(1.47 \pm 0.29) \times 10^7$ (1 ~10^5
Odessa [IA iron]	Feldspar‡ [1]	~10^5 [cosmic rays]	0) $(5.47 \pm 0.43) \times 10^6$
Odessa [IA iron]	Diopside§ [3]	$(9.05 \pm 0.27) \times 10^6$ [fission of ^{238}U and ^{244}Pu]	

* Etchant, boiling WN[5] solution at *p*H 8 for 2 or 1 h.
† Etchant, 60 wt % boiling aqueous NaOH sc or 1 h 40 min.
‡ Etchant 40 wt % boiling aqueous NaOH sc g aqueous NaOH soluti
§ Etchant as for feldspar + 30 min in 60 wt %
All etching stages carried out under reflux.
‖ Track densities measured using Leitz ort optical microscope at 6 olivine which were meas magnification, except for shallow pits in Brer tion. The SEM was also us under Cambridge Mk IV SEM at 5,000 × mag es.
check on the integrity of metal–silicate boun

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g electron micrograph showing the boundary
eft hand side) and a diopside crystal (right hand
m the Odessa iron. The slice was first etched in
NaOH solution for 100 min to develop tracks in
n diopside were then revealed after a further
in 60 wt % NaOH solution. The scale bar
ere is no indication of a very high track density
of the disintegration which would result from
tensely radiation damaged boundary region.

retei

diops 3. Fractionation trends for Pu and U in
upper known, however, if we take an extreme
track 0.5 at 4.58×10^9 yr ago then the diopside
$\sim 4.2 \times$ a still imply track retention as long ago as
tion of esponds to ~4 half lives after the forma-
with Rh for the postulated SHE if we assume
concenti life of $\sim 10^8$ yr. With an initial SHE
track ret kg kg^{-1} in the metal phase then at this
ing. If this would be $\sim 10^{-5}$ kg kg^{-1} still remain-
number cayed to extinction via fission then the
boundary s crossing 1 cm^2 of a metal–silicate
on the silic 10^{13}. (The number of tracks per cm^2, ρ,
approximat contact with the metal will be given
fraction of $S\Omega(CN_0R/A)$ where C is the weight
g cm^{-2}, N_0 is tal, R the fission fragment range in
the SHE.) Thi umber and A is the atomic weight of
to render a ~1 ous track density probably sufficient
to observe trac f silicate totally amorphous[9]. Failure
fore implies that t the level of $\sim 10^6$–10^7 cm^{-2} there-
metal was at leas ng superheavy content of the Odessa
inferred from trac ders of magnitude lower than those
take as the SHE a data by Libby *et al.*[4]. If instead we
$\sim 4.2 \times 10^9$ yr ago (that estimated for the lunar core at
we arrive at an e of ref. 4) that is, $\sim 10^{-7}$ kg kg^{-1} then
10^{11} cm^{-2}, still five boundary track density of $\sim 5 \times$
would be compatible f magnitude above a level which
In the case of Ode e observations reported here.
acks implies that no side, observation of ^{244}Pu fission
uld remove the SHE ling event can be invoked which
^{244}Pu fission tracks. track excess as this would remove
10^8 yr then a therma urse, if the SHE had a half life of
uld remove the SHE ory could be constructed which
ks intact. Presumably s but leave the bulk of the ^{244}Pu
h would drive a luna h SHEs could not then be those
on years. The SHE abu re dynamo for several hundred
ances could be made compatible

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with the observations provided that we accept a somewhat contrived model, namely that the silicates and metal were not placed into intimate contact until $\sim 10^9$ yr ago. Radiometric ages for the silicate inclusions in irons are generally $\sim 4.6 \times 10^9$ yr (ref. 10) and none is yet known to be younger than 3.8×10^9 yr. Such an emplacement event therefore would have to be such that the Rb–Sr, K–Ar ages were not reset.

Taking as a generous estimate for the upper limit to the boundary track excess the actual measured boundary track densities of Table 1, column 5, then the corresponding upper limits to the concentration of fissioning SHEs become $\sim 2 \times 10^{-15}$ kg kg^{-1} for the metal phase of Brenham (from the density of deep etch pits) and $\sim 10^{-12}$ kg kg^{-1} for metal adjacent to Odessa diopside. These figures apply to the time at which quantitative retention of fission tracks began in the silicates. This is probably not later than $\sim 4.2 \times 10^9$ yr ago in the case of Odessa diopside but this is not known with any certainty for the case of Brenham olivine. Of course, the presence of α-decaying superheavy elements is not precluded by these data but if α-decay is the predominant mode of decay for the SHE then the similarity between trace element abundance peaks and fission product abundance curves pointed out by Libby *et al.*[4] must be purely fortuitous.

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Amino acids in the Yamato carbonaceous chondrite from Antarctica

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More than one thousand pieces of meteorites have been discovered recently in Antarctica[1–4] which were preserved in the Antarctic Ice Sheet since their fall and were brought out on to the surface of the bare ice. They are likely to have the least possible terrestrial contamination, thus providing a new opportunity for the study of primordial organic synthesis in the early solar nebula[5] and of chemical evolution before the emergence of life[6,7]. Organic compounds of extraterrestrial origin were first detected in the form of protein and non-protein amino acids with an equal abundance of their D- and L-enantiomers in the Murchison carbonaceous chondrite[8,9]. This ratio of enantiomers has been used as a criterion for the meteoritic origin of the amino acids detected subsequently in other meteorites[10–13]. We present here detailed evidence for the presence of amino acids of extraterrestrial origin in our Antarctic carbonaceous chondrite[14].

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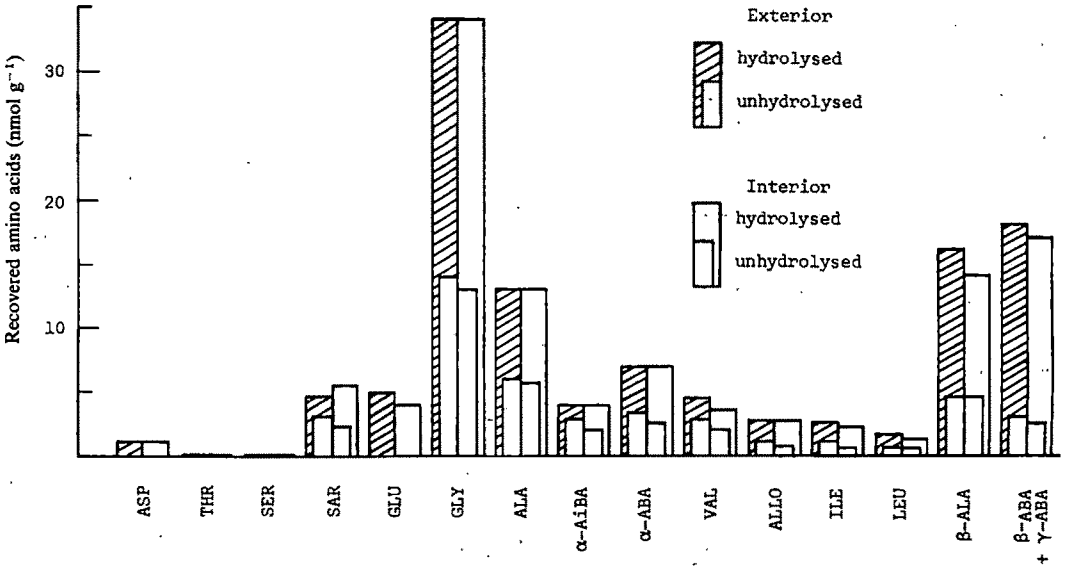


Fig. 1 Amino acids recovered from the Yamato meteorite 74662. The quantities were estimated by the amino acid analyser. The amount of β-ABA + γ-ADA consists mostly of γ-ABA by the gas chromatographic study.

A ~5-g fragment of the original 150-g meteorite was separated into exterior, middle, and interior portions. These portions were pulverised and nearly 1 g of the exterior and interior was individually extracted with water at 105 °C for 24 h. The water extracts were centrifuged and the supernatants were recovered. The residues were rinsed twice with water and combined with the corresponding supernatants. Half of each resulting solution was further halved into two aliquots. One of the aliquots was reduced to an appropriate volume and analysed by a Durrum 500 amino acid analyser. The other aliquot was dried under an IR lamp and derivatised, first, with isopropyl alcohol, 1.5 M HCl, and, then, TFAA-CH_2Cl_2 (1 : 2 by volume). The N-TFA-isopropyl-derivatives of amino acids were analysed by a gas chromatograph (GC) equipped with a Chirasil Val glass capillary column (25 m) using a nitrogen detector. The other half of the resultant solution was evaporated and was hydrolysed with 6 M HCl at 105 °C for 24 h. The hydrolysed solution was divided into two equal portions which were dried completely under the IR lamp. One of the dried samples was redissolved with an appropriate volume of water and analysed by the amino acid analyser. The other dried sample was subjected to the derivatisation for the enantiomer study by GC. All analytical preparations were conducted in a class 100 clean room.

Fifteen amino acids were detected in the Yamato meteorite: of these, nine are protein and six are non-protein amino acids. The individual amino acids have abundances between 34 and <1 nmol per g meteorite. Figure 1 compares the exterior versus interior and the unhydrolysed versus hydrolysed portions. The abundances in the exterior and interior are similar. The yields of amino acids increase two to three times after the acid hydrolysis.

The gas chromatogram obtained with the nitrogen detector shows a complex mixture. However, the D- and L-enantiomers of alanine, aspartic acid, and glutamic acid have nearly equal abundance. If other overlapping peaks around the baseline level are subtracted this fact might become even more evident (Fig. 2). The nearly equal abundance was observed in all the unhydrolysed and hydrolysed portions of the exterior and interior of the meteorite.

The amino acid assemblage, their concentrations and the enantiomeric ratios found are indicative of amino acids of abiotic origin and, therefore, extraterrestrial in nature. It may be argued that the observed DL ratios were produced during the preservation of the meteorite after contamination with terrestrial biological material at the time of fall. Unlike relatively recent falls of the Murchison (1969), Murray (1950), Orgueil (1864) and Mighei (1889) that exclude a pronounced racemisation during the terrestrial age, Antarctic meteorites might have enough time for an extensive racemisation. The age of the present bare ice surface of the Yamato Mountains may be 240,000 yr (ref. 2). However, as these meteorites fell on, and were subsequently preserved in, the Antarctic ice the terrestrial contamination would be insignificant and the racemisation process must have been exceedingly slow. The presence of non-protein amino acids also clearly indicates prebiotic synthesis. Further, the remarkable similarity in the amino acid abundance of the exterior and interior suggests that the terrestrial organic contamination was minimal.

More confirmation is provided by the equal abundance of D- and L-enantiomers of protein amino acids such as alanine, aspartic acid and glutamic acid in both the exterior and the interior. A similar study of the Mighei meteorite showed a striking L-enantiomer predominance of protein amino acids in its exterior portion, which diminished towards the interior of the

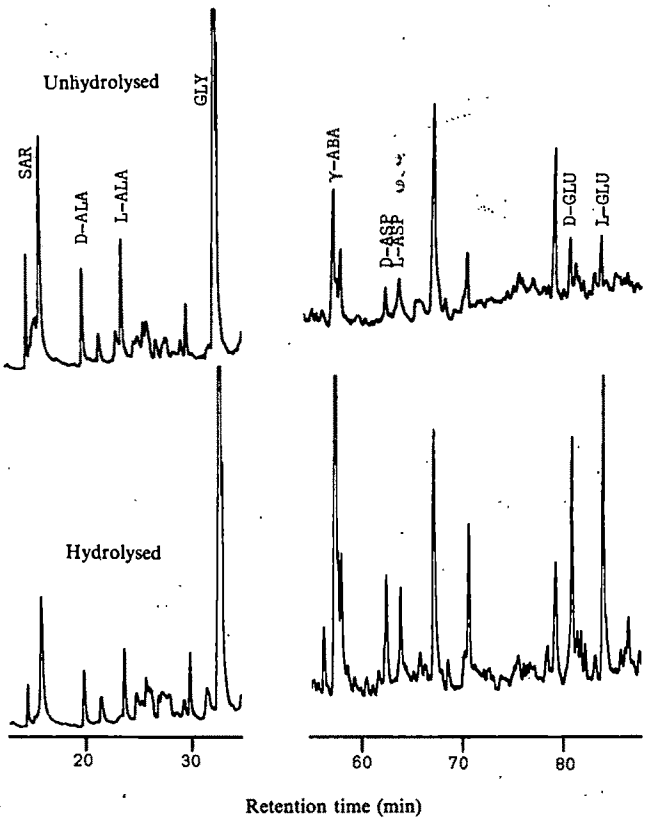


Fig. 2 Gas chromatograms of amino acids (N-TFA-isopropyl esters) of the interior portion of the Yamato meteorite 74662.

meteorite. The non-protein amino acids, however, did not show a difference throughout the exterior to interior portions[15].

The Yamato meteorite contains amino acids similar to those in the Murchison and Murray meteorites. All three are type II carbonaceous chondrites. The abundance of amino acids are also of the same order of magnitude. Glycine, the most abundant amino acid, is 34 nm in the Yamato, 81 nm in the Murchison[8,10,11], and 40 nm in the Murray[11]. Another common feature is the marked increase in the yields of aspartic acid and glutamic acid (both dicarboxylic amino acids) after acid hydrolysis. The Murchison and Murray meteorite also have aspartic acid and glutamic acid in very small quantities in the unhydrolysed portion[11]. This resemblance minimises the possibility that these amino acids in the unhydrolysed portion of the Yamato meteorite had been washed out by water on the surface of bare ice because dicarboxylic amino acids are anionic under a neutral *p*H. The observation of the recovered body of the Yamato meteorite indicated that about half of the fusion crust was lost by weathering[14]. Nevertheless, the metoerite does not seem to have been significantly altered by weathering. It seems that only the portion of the crust, physically broken from the parent body, was lost during its period on Earth.

The Allan Hills carbonaceous chondrite, also from Antarctica, exhibits an equal abundance of the D- and L-enantiomers in the exterior and interior[16]. Although the amino acid concentration was much lower in the Allan Hills meteorite than in the Yamato meteorite, these two Antarctic carbonaceous chondrites seem to be the least contaminated of the meteorites we have examined so far.

Since the fall of the Murchison meteorite, information on organic compounds indigenous to carbonaceous chondrites has revealed the processes of primordial organic synthesis. Studies of Antarctic carbonaceous chondrites, such as the Yamato 74662 which contains organic material of nearly the same concentration level as that of the Murchison meteorite, provide additional information useful for the understanding of extraterrestrial organic material.

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Quantification of monocarboxylic acids in the Murchison carbonaceous meteorite

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Although the nature and origin of carbon and its compounds in meteorites have been reviewed by Hayes[1] and Nagy[2], much of our understanding of carbon and its compounds in carbonaceous chondrites remains speculative. Evidence for the extraterrestrial nature of the solvent-soluble organic matter was first found in the Murchison carbonaceous chondrite[3]; this material in the Murchison meteorite and in other meteorites has been identified as amino acids and precursors[3–10], monocarboxylic acids[11], dicarboxylic acids[12], hydrocarbons[6,13,14], amines[15], and other organic compounds[16–20]. The data suggest a chemical synthesis from simple precursors. Although the mechanisms by which these organic compounds were formed are unknown, theories (such as Fischer-Tropsch Type (FTT)[21] and discharge reactions[22]) have been proposed to account for their presence. Knowledge of the distribution of isomers in these chondrites is essential for understanding the mechanism of synthesis, and we report here the quantification of some of the branched- and straight-chain isomers of the monocarboxylic acids extracted from the Murchison meteorite.

The successful use of deuterated analogues for the quantification of amino acids[23] led to the selection of this approach for the quantification of the monocarboxylic acids. The deuterated and undeuterated monocarboxylic acid solutions for calibration and spiking purposes consisted of the 11 acids listed in Table 1. The deuterated analogues were prepared by the method of Pfeffer and Silbert[24].

A 1.084-g sample of finely crushed Murchison (interior) was refluxed for 3 h in a KOH–methanol solution (0.5g : 8 ml). After cooling to room temperature, the methanolic solution with some suspended solids was decanted into a Teflon test tube and the residue was washed twice with 5-ml portions of distilled water. After the combined solutions had been centrifuged, the supernatant was transferred to a 25-ml round-bottomed flask and the solvents were removed under reduced pressure. The resulting residue was acidified to *p*H 1 by adding concentrated hydrochloric acid dropwise, while cooling the flask in an ice-water bath. The aqueous mixture was filtered to remove inorganic salts and the filtrate collected in a screw-cap vial. The inorganic salts were rinsed with 3.0 ml of diethyl ether and collected in the same vial that contained the aqueous acid solution. After shaking for 1 min, a sample of the ether phase was analysed by gas chromatography using a 46-m-long stainless steel column with an i.d. of 0.05 cm; the column was coated with UCON 550-H_3PO_4. Comparison of this gas chromatogram with that of a standard solution of deuterated carboxylic acids prescribed the quantity of the deuterated solution to be added to the meteorite extract in order to have approximately a 1 : 1 correspondence in molar concentration between the carboxylic acids in the meteorite sample and their deuterated analogues. After adding the solution of deuterated acids and intimate mixing, the ether layer was separated from the aqueous phase and the latter was extracted three times with 1-ml portions of diethyl ether. The combined ether extracts were dried over sodium sulphate, filtered and concentrated to a small volume (~50 μl) for analysis by combined gas chromatograph–mass spectrometry (GC–MS).

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Table 1 Monocarboxylic acids in Murchison meteorite

Compound	Abundance (μmol g^{-1})	$N/\Sigma Br$
Ethanoic	1.03 ± 0.06	—
Propanoic	1.83 ± 0.03	—
2-Methylpropanoic	0.50 ± 0.06	0.76
Butanoic	0.38 ± 0.02	
3-Methylbutanoic	0.09 ± 0.02	
2-Methylbutanoic	0.12 ± 0.02	0.57
Pentanoic	0.12 ± 0.03	
4-Methylpentanoic	0.07 ± 0.02	0.86
Hexanoic	0.06 ± 0.01	
Heptanoic	0.03 ± 0.01	
Octanoic	0.01 ± 0.002	

To determine the effectiveness of this procedure to extract the monocarboxylic acids from the sample, the solid meteorite residue was again pulverised and re-extracted by the procedure described. No monocarboxylic acids were found in the second extract, which strongly suggests that the first extraction process had completely removed these acids from the sample. Therefore, the values for the monocarboxylic acids (Table 1) are good estimates of the abundance of these compounds in this sample of the Murchison meteorite. Satisfactory procedural blanks accompanied all the experiments.

An area of concern is the possible degradation of these saturated monocarboxylic acids during extraction. However, the potassium hydroxide and refluxing methanol involved, are also used in a procedure to identity these acids[25] and are not known to degrade them[26]. It is reasonable to assume that the saturated monocarboxylic acids should survive a similar treatment.

Our procedure was similar to that used previously but with slight modification[23]. Mass spectra were acquired every 4.2 s over the mass range 12–400 throughout the GC run. Identity of the individual components was established by evaluation of the mass spectra obtained for each component eluting from the GC. Quantification was accomplished by selection of mass spectral ions of the corresponding deuterated and undeuterated analogues of each monocarboxylic acid and measurement of the area ratio of each m/e pair within a given gas chromatographic peak. The results of this measurement from the deuterium-spiked Murchison meteorite extract were then compared with standard calibration curves obtained in an analogous fashion from standard mixtures. The quantification data for the monocarboxylic acids reported in Table 1 were obtained in this way.

As an extraterrestrial chemical synthesis seems to be responsible for the presence of the monocarboxylic acids in the Murchison meteorite, a similar distribution of decreasing concentration with increasing molecular weight would be expected to that found for the amino acids[9]. Such a distribution for the monocarboxylic acids was not obvious from the data previously reported[11]. In that study, the GC peak heights (and area) for propanoic and hexanoic acids appeared to be equal, suggesting equal concentrations of these two compounds. In reality, propanoic acid is 30 times more abundant than hexanoic acid (Table 1). Gas chromatogram peak areas do not adequately reflect the distribution of components, as these results could be an artefact of the analytical procedure used. For example, the distribution of the acids previously observed could have been skewed by the differences in partition coefficients of these acids in the solvents utilised. Thus, the quantification data presented in Table 1 reflect more accurately the monocarboxylic acid distribution in that the deuterated analogues added to the meteorite extract during the isolation procedure would be subjected to the same analytical skewing.

The monocarboxylic acids are present in the Murchison meteorite in higher concentration than are the amino acids, which are the only other group of compounds that have been rigorously quantified[5,10,23]. The concentration of propanoic acid (1.83 μmol per g of meteorite), the most abundant monocarboxylic acid, is ~58 times greater than the most abundant amino acid, glycine (0.041 μmol g^{-1})[23] found in aqueous extracts of the Murchison meteorite. We also note a general decrease in concentration of the monocarboxylic acids as the number of carbon atoms in these acids increased, a trend similar to that previously noted for the amino acids[9]. This supports the hypothesis that monocarboxylic acids, like amino acids, are present as the result of an extraterrestrial abiotic synthesis. Table 1 shows that nearly equal concentrations of each branched- and straight-chain isomer with the same carbon number are present in the sample.

Miller[27] showed that formic, acetic and propanoic acids could be synthesised when a primitive-Earth-type reducing atmosphere was subjected to a spark discharge. Similarly, C_2–C_{12} monocarboxylic acids have been produced when a methane–water mixture was exposed to a semicorona discharge[28]. In the discharge experiment monocarboxylic acids with carbon numbers less than six were present both as straight- and branched-chain isomers, but for those acids with carbon numbers of six or greater, only the branched-chain isomers were observed[28]. Since the Murchison meteorite contains straight-chain monocarboxylic acids with carbon numbers greater than five (hexanoic, heptanoic and octanoic acids) a re-evaluation of the laboratory simulation experiment is warranted. An alternative mechanism (FTT) has been proposed as the possible pathway to the organic compounds in meteorites[21]. Theoretical consideration of the proposed mechanism for the FTT process predicts a high concentration of straight-chain isomers, with $N/\Sigma Br > 1$, for any given carbon number (N, straight-chain isomer concentration; Br, branched-chain isomer concentration). Recent FTT experimental studies in which monocarboxylic acids were synthesised[29] support this prediction.

Although Leach *et al.*[29] were not able to quantify the C_2–C_6 monocarboxylic acids which would have allowed a direct comparison of their results with those reported in Table 1, they did report quantification of the C_7–C_{18} monocarboxylic acids that were synthesised by the FTT process. Straight-chain isomers were observed to predominate over the branched with $N/\Sigma Br$ ratio falling between 1.7 and 5.

In contrast, the predominance of straight- over branched-chain isomers is not observed for the isomeric monocarboxylic acids found in the meteorite extract ($N/\Sigma Br < 1$, Table 1). Moreover, this study and previous work[11] indicate that other branched-chain isomers are present. If these were included in the quantification, the value of $N/\Sigma Br$ would be further decreased. Thus, if the monocarboxylic acids present in the Murchison meteorite are the original, unchanged, extraterrestrially synthesised compounds, its isomer distribution is not consistent with that of the FTT process. Thus the FTT process, conducted in reported experimental conditions[29], is not apparently singly responsible for the formation of the acids found in the Murchison meteorite. If a two-step mechanism is envisaged, a primary FTT reaction followed by a secondary partial equilibration[21], then the effect of the equilibration step on product distribution deserves careful examination. It has been predicted on theoretical grounds[30] that there would be little difference in concentration between straight- and branched-chain isomers of the short-chain (C_2–C_8) monocarboxylic acids formed inorganically in equilibrium conditions. The meteorite results reported here apparently support this prediction with regard to isomer distribution. Our results are consistent with the production of the monocarboxylic acids by a naturally occurring chemical synthesis and support the chemical evolution theory.

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Experimental evidence of rare earth element immobility in greenstones

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Significant petrogenetic importance has recently been placed on the Nd isotopic composition and variable rare earth element (REE) content of basalts, although the effect of alteration on the REE has not been fully investigated. REE analyses of oceanic[1–6] and ophiolitic basalts[7,8] suggest that the light REE (La, Ce, Nd, Sm, Eu) are mobile during basalt–seawater interaction at relatively low temperatures. However, the behaviour of the trace and rare earth elements during greenstone formation at higher temperatures is less well understood[9–15], primarily because of uncertainties regarding the composition of the unaltered precursor, temperature, pressure and effective water/rock ratio. In an attempt to overcome these uncertainties we have analysed basalt glass subjected to 10 individual basalt–seawater interaction experiments. In all these experiments the REE are found to be immobile at temperatures of 150–350 °C even when the basalt is totally altered to clay. Furthermore the data suggest that the minor fluctuations observed in the light REE content of some greenstones[9,10] may result from a low temperature overprint due to retrograding of the greenschist facies basalts.

Details of the experimental, analytical and sampling procedures used in this study have been described elsewhere[8,15,16]. Briefly, experiments were performed at temperatures from 150 to 350 °C, 500 bar and water/rock mass ratios of 10–125 using Dickson hydrothermal equipment[16], and REE were analysed by instrumental neutron activation analysis (INAA). Figure 1 shows the REE composition of the fresh basalt glass (*a*) and experimental alteration products relative to chondrite (*b–e*).

The glassy tholeiite (that is, unaltered precursor) has a light REE depleted profile with a detectable Eu anomaly—features characteristic of many ocean-floor basalts. All of the experimentally altered basalts have similar REE profiles, although

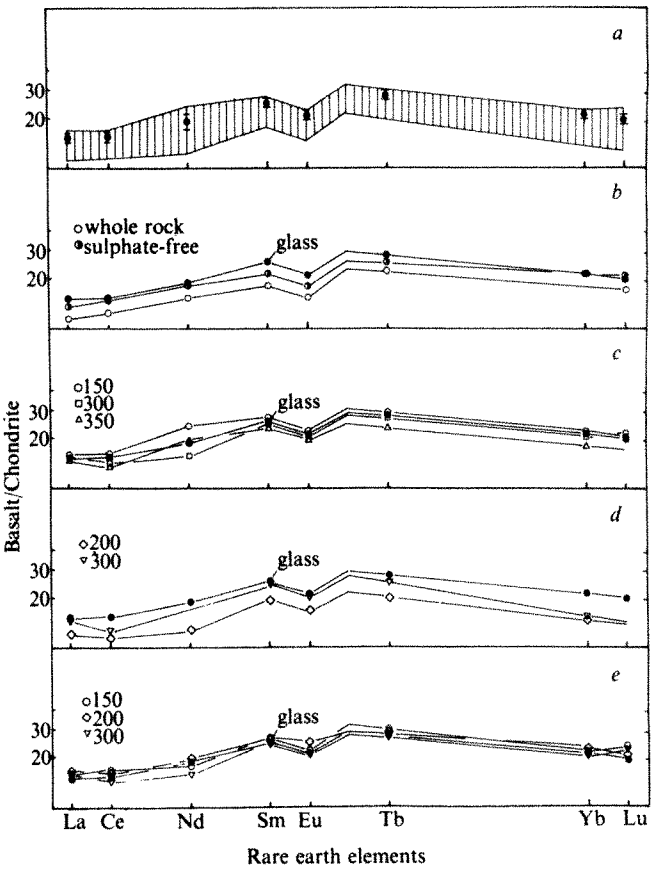


Fig. 1 Chondrite normalised REE patterns of experimentally altered basalt and the unaltered precursor. The figure includes the REE abundance profile of *a*, the fresh glass (●) with error bars and the total range in REE produced by alteration of this glass at 150–350 °C and a water rock ratio (W/R) of 10–125; *b*, the whole rock altered at 300°C and a W/R 125 before and after correction for 20% modal anhydrite; *c*, the whole rock altered at 150–350 °C and a low W/R of 10; *d*, the whole rock altered at 200–300°C and a higher W/R ratio; and *e*, the silicate fraction (chlorite + smectite) altered at 150–300 °C and a W/R of 62 (anhydrite was removed by repeated washings in deionised water[15]).

they differ in their absolute REE content. We believe this to be a dilution effect caused by the presence of anhydrite, a phase ($CaSO_4$) which precipitated during the experiments[15]. For example, anhydrite constituted approximately 20% of the 'bulk' alteration from interaction at 300°C and a water/rock ratio of 125. Anhydrite contains an insignificant amount of REE, so its presence simply and effectively dilutes the REE abundances of the alteration products as a whole. Thus correcting for the presence of anhydrite, a REE profile develops which is identical to that of the unaltered basalt (Fig. 1*b*). This is highly significant since the basalt was entirely transformed to a mixed layer chlorite–smectite phase. This was the case for all silicate fractions (altered basalt minus anhydrite)[15] separated from the altered solids from each experiment. Thus, for the duration of these experiments, REE were conserved and in general revealed a solubility similar to previously defined conservative species, such as Co, Cr, Ni, Al, V and Ti (W.S. and M.J. Mottl, unpublished).

Hydrothermal alteration of the oceanic crust and greenstone formation apparently occurs at temperatures, pressures, and water/rock ratios analogous to those used in these experiments. The immobility of REE in greenstones from the Point Sal[8] and Troodos[11,19,20] ophiolites, and the Mid-Atlantic[10] Ridge compares favourably with the data presented here. Similarly, amphibolites[21] and chlorites[22] from the seafloor exhibit REE abundances typical of oceanic basalts. In the case of the chlorites

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it was proposed that they represent disrupted fragments of submarine greenstones[22]. This conclusion is certainly compatible with the results of the present investigation which confirm the relative immobility of REE during hydrothermal processes (Fig. 1), hence, their use as a 'fingerprinting' tool to deciphor origins of recent and Archean[9,23] greenstones.

However, it is difficult to reconcile the fluctuations in light REE content of some greenstones with the experimental data. Because the light REE are more mobile at low temperatures than at 150–350°C the apparent mobility of the light REE in rocks with greenschist facies mineralogy may partly reflect retrograde reactions. Such reactions would superimpose a low temperature overprint on the greenstone thus modifying the mineralogy and chemistry of the rock.

Massive volumes of seawater must be involved in retrograde processes if these events are to substantially modify the REE content of basalt. Furthermore, that LREE are specifically enriched during this event suggests ionic size–charge relationships may be important if not restrictive to secondary REE enrichment. Certainly this is true for post-greenstone enrichment of K and Rb. These species are apparently incorporated in cation exchange sites of clay minerals (smectite, mixed layer chlorite–smectite, chlorite) in greenstones. Sediments found within the Troodos ophiolite have a La, Ce, and Nd content 10–100 times that of basalt[7]. Similarly, clay rich umbers[7] (ferromanganoan oxyhydroxides and silicates) concentrate the light REE (except Ce) relative to basalt. Thus the light REE can be enriched in clay minerals within greenstones provided adequate exposure to seawater is maintained for prolonged periods of time (>1 Myr). This is particularly noticeable in basalts found within or adjacent to the discharge zones of the Troodos submarine geothermal system[7]. The basalts are characterised by pronounced negative Ce anomalies and enhanced La and Nd contents, whereas, zeolite and greenschist facies[11,20] basalts isolated from abundant seawater show no such REE mobility.

The effect of availability and accessibility of seawater and REE mobility is also amply illustrated in the Point Sal[8] ophiolite, Caifornia. At Point Sal the upper smectite bearing lavas have been exposed to seawater for ~15 Myr before deposition of a sedimentary horizon primarily consisting of chert[26]. Thus, these volcanic flows were actively and effectively altered by seawater for approximately 15 Myr before being isolated. The light REE in these lavas were mobilised and are now characterised by negative Ce anomalies[8]. Similar anomalies have been observed in seawater[27], marine precipitates[7,27], and basalts[2,4,7] exposed to low temperature alteration or retrograde reactions. However, at a stratigraphically lower level in the Point Sal ophiolite, greenstones show little or no change in REE abundance even though these rocks are thoroughly altered and characterised by abundant epidote and quartz (epidosite)[8]. We believe that the apparent absence of REE mobility during greenschist facies metamorphism results from limited exposure of these rocks to fluids (seawater) at low temperatures. Vein mineralisation during incipient alteration may have effectively reduced the permeability of these rocks to the point of not permitting access of seawater subsequent to the primary alteration event.

Variations in the light REE content of greenstones relative to an inferred unaltered precursor have been noted[9,10,24]. These changes may in part reflect alteration effects subsequent to the metamorphic event which initially transposed the basalt to greenstone. The suggestion that greenstones are modified chemically subsequent to their formation is supported by the light REE enrichment noted and the trace element abundances reported for these rocks. It has been shown experimentally that K is leached from basalt[15] reacted with seawater at temperatures ≥150°C; whereas, at lower temperatures the reverse is true, that is, K is removed from seawater and enriched in the altered basalt[25].

The above discussion emphasises that metamorphic minerals produced during greenstone formation immobilise the REE and that changes in the light REE may result from retrograde effects. There remains the problem of spilites and greenstones with modified ΣREE contents. The origin of these rocks is clearly complex, although Sun and Nesbitt[9] and Hellman and Henderson[13] attribute the consistent change in REE profile to alteration processes. However, the REE content of an altered rock is influenced by, (1) the chemistry of the unaltered precursor, (2) the extent of igneous fractionation[28], and apparently (3) the degree of retrograde reactions. Thus the REE chemistry of these rocks[9,13] probably represents a complex interplay of primary and secondary processes, and their origin is at best equivocal.

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A new date for *Ramapithecus*

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Recent work in the Middle Siwalik rocks of the Potwar Plateau in northern Pakistan is doing much to further our understanding of Miocene mammalian evolution, in particular the evolution of *Ramapithecus* and *Sivapithecus*, hominoids considered important for human ancestry[1]. It is crucial to the understanding of hominoid evolution to place the fossils in as precise a chronologic framework as possible. As there is a lack of material suitable for radiometric dating, it is necessary to rely on the correlation of the magnetic stratigraphy associated with the Siwalik faunas to the calibrated magnetic polarity time scale (MPTS). Initial age estimates by Barndt *et al.*[2] were based on reconnaissance level palaeomagnetic sampling. The more detailed sampling presented here suggests a revised correlation of the local magnetic stratigraphy to the time scale and hence a revised age estimate for *Ramapithecus*: 8 Myr now seems more likely than the previous estimate of 9 Myr.

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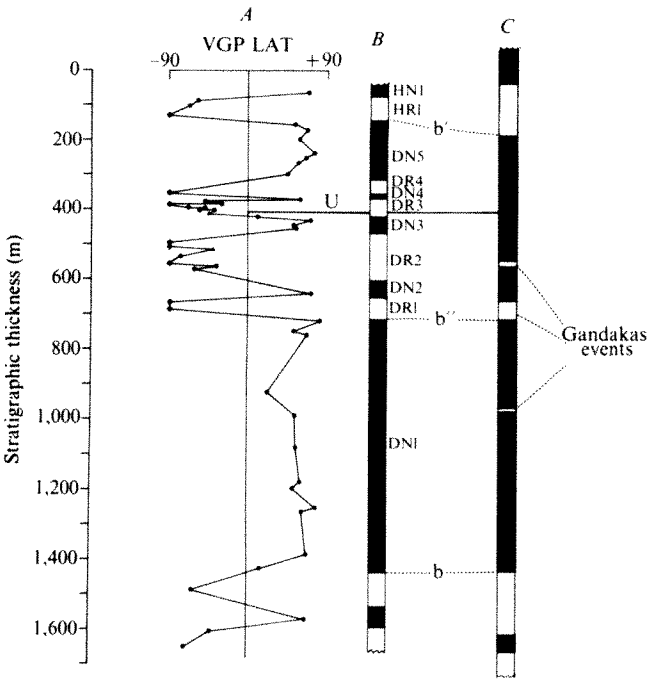


Fig. 1 Column *A*, VGP latitudes calculated from site means of the Ratha Kas section[1]. Class A, B and C data (see text) are denoted by circles, triangles and diamonds respectively. Column *B*, Block diagram of section in (*A*), black is normal polarity, white is reversed. Magnetozones D and H stand for Dhurnal and Hasal chronostratigraphic units[12]. N and R stand for normal and reversed respectively. Column *C*, Part of the magnetic stratigraphy of Barndt *et al.*[2] correlated to section shown in (*A*) and (*B*) by means of stratigraphic marked bed U.

Barndt *et al.*[2] established a local magnetic stratigraphy which was correlated to the MPTS based on various faunal constraints and later showed it to be consistent with a dated bentonite[3]. The bulk of the hominoid material was thus estimated to be around 9 Myr old. In the work described here, part of the magnetic stratigraphy of Barndt *et al.*[2] was examined in great detail, originally to precisely define an isochron within the sediments that could be used for lateral correlation of hominoid levels. However, as a result of this more detailed work it became clear that the original identification of the magnetic reversal sequence needed revision.

The location, geological setting and stratigraphy are described in detail elsewhere[4]. Briefly, the strata exposed on the northern flank of the Soan Synclinorium are cut by rivers flowing approximately perpendicular to strike. The sampling technique used was similar to that described by Johnson *et al.*[5] which is ideally suited for Siwalik sediments. Sampling of river channel exposures resulted in the section shown in Fig. 1. Each site consists of three to five samples and the sites were placed in a stratigraphic section constructed by members of the joint GSP–Yale University field party. The section presented in Fig. 1 consists of 81 sites in 1,600 m for an average site density of about 1 in 20 m. The actual site density is dependent on the relative abundance of sandstone (unsuitable for palaeomagnetic purposes) and the finer grained units, and thus varies over the thickness of the section. The maximum separation is estimated to be about 100 m and the minimum separation is about 1.5 m.

To allow unambiguous correlation of the magnetostratigraphic section of the Khaur area, a major sandstone unit, referred to here as the 'U' sandstone, was carefully traced in the field from section to section. The magnetic stratigraphy presented here can thus be precisely correlated to the sections of Barndt *et al.*[2].

An extensive study of the magnetic mineralogy of Middle Siwalik rocks has been completed, including a conglomerate test coupled with rock magnetic studies which will be presented elsewhere. It is reasonable to assume that the primary remanence of the rocks is carried by haematite, was acquired during or soon after deposition, and can be isolated by thermal demagnetisation. After thermal demagnetisation at 550 °C, site means were calculated using Fisher statistics[5]. Sites found to be statistically significant by Watson's test for randomness[7] (for a mean of three sample directions of unit length, the resultant vector length must be ≥ 2.62) after thermal cleaning are designated Class A. Statistically significant sites after a.f. demagnetisation at peaks of 300 Oe are Class B, useful primarily when the specimens disintegrate on heating. Class C are sites with two specimens showing reversed directions in good agreement where the third is either lost, crumbled or widely deviant. Class D are samples showing a strung distribution to the South and are thought to be possibly reversed. Only Class A, B and C data are used here. Class A and B site means were converted to virtual geomagnetic pole (VGP) latitudes.

In Fig. 1, Classes A, B and C are represented by circles, triangles and diamonds, respectively. In some cases sites showing a statistically significant normal cluster after a.f. treatment became reversed after thermal cleaning. To ensure the best quality palaeomagnetic data, only Class A data are used for normal polarity information as there is mounting evidence to indicate that a.f. demagnetised specimens have not been properly cleaned. Each labelled magnetozone of Fig. 1 has been substantiated in at least three stratigraphically correlatable sections in the Khaur area, strengthening the assumption that they represent palaeomagnetic field behaviour.

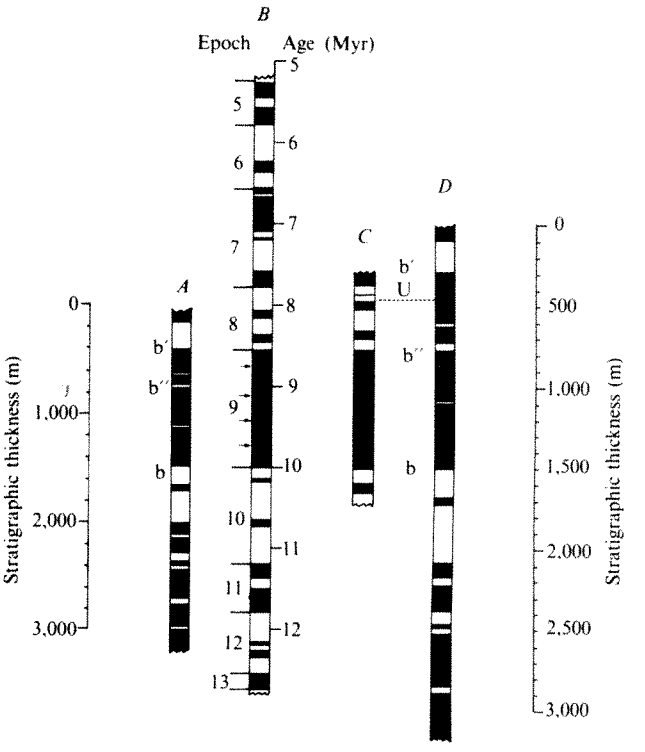


Fig. 2 Correlation of the magnetic stratigraphy to the magnetic polarity time scale. Column *A*, Previous correlation to the time scale of the Barndt *et al.*[2] section. Column *B*, The revised dates given by Mankinen and Dalrymple[13] for the time scale of LaBrecque *et al*[14]. Column *C*, The section presented in this paper drawn to scale at right assuming DN1 (see Fig. 1) is Epoch 9 of the time scale. Column *D*, The section in (*A*) redrawn at the same scale as the section in (*C*). The stratigraphic marker bed U allows unambiguous stratigraphic correlation of the Barndt *et al*[2]. section in (*D*) and the more densely sampled section of (*C*). The bulk of the primate material has been found in close association with the U marker bed and thus is about 8 Myr old.

The magnetic stratigraphy of Barndt *et al.*[2] established the existence of a long zone of dominantly normal polarity (b to b′ of column *C* of Fig. 1) which was apparently punctuated by several reversed events known as the 'Gandakas Events'. The Barndt *et al.*[2] section was correlated to the MPTS as shown in Fig. 2*A* based on faunal constraints and visual correlation. The magnetic stratigraphy in Fig. 1 represents a more densely sampled section of the same strata and it is clear that the Gandakas Events represent more reversed time that was previously supposed.

Several assumptions must be made to make a useful correlation of the magnetic polarity stratigraphy to the MPTS. These include: (1) an estimate of the range of ages that the section could encompass; (2) that for a given lithofacies, when averaged over a large stratigraphic thickness, thickness is linearly proportional to time; (3) the section is densely sampled enough to assume that it approximates field behaviour; and (4) the magnetic remanence of the rock was acquired shortly after deposition and represents a record of the magnetic field at the time of formation of the rock. These assumptions are not always valid and great care must be taken to substantiate them whenever possible.

Both Barndt *et al.*[2] and the magnetic stratigraphy of this paper are bounded by the following palaeontological criteria. A maximum age for the bottom of the section is given by the sudden appearance of the extinct equid, *Hipparion*, within the Khaur normal zone (DN1 of Fig. 1). The earliest Old World *Hipparion* has been dated[8] at 12.5 Myr, and the Indian *Hipparion* is probably younger (B. MacFadden, personal communication). A minimum age for the top of the section is given by the conspicuous absence of hippopotamus, *Hexaprotodon*, which occurs suddenly and abundantly in the Upper Siwaliks. The oldest hippopotamus in the Siwaliks is found in the Rhotas section at the Gilbert/Epoch 5 boundary. The absence of *Hexaprotodon* (John Barry, personal communication) in the section presented here and in that of Barndt *et al.*[2] therefore suggests that the top of the section is probably older than about 5 Myr.

Based on the more detailed section presented here it seems unlikely that the Gandakas Events (Fig. 1*C*) are all within Epoch 9 because (1) in the more detailed section shown in Fig. 1, the reversed zones represent a large stratigraphic thickness and it is unlikely that such long reversed zones would be unresolvable in the seafloor spreading record, and (2) the four events or intensity fluctuations discussed by Blakely[10] and Cande and Labrecque[11] are evenly spaced within anomaly 5 (Epoch 9) and the reversed zones in Fig. 1 are tightly grouped. A better correlation of the magnetic stratigraphy of the Khaur area, based on the work presented here, is shown in Fig. 2*C* where the vertical scale is expanded by assuming the two upper Gandakas Events to comprise Epoch 8. Epoch 9 is thus b to b″ of Fig. 2. Figure 2*D* shows the Barndt section redrawn to the same scale as Fig. 2*C*. Clearly the overall correlation is much improved and there is now a reasonable fit to the time scale from the top of Epoch 8 to the bottom of Epoch 11. Below Epoch 11 there seems to be a dominance of normal polarity which seems to correlate with an increase in red pigmentation. Recent efforts using thermal demagnetisation techniques have improved the fit below Epoch 11 dramatically (to be presented elsewhere).

Fig. 1 suggests three short normal subzones, DN2-DN4, within the dominantly reversed zone. Since the normal subzones occur in correlated sections of different rock types and persist after thermal demagnetisation, these are thought to be real. The uppermost zone, DN4, was ignored in the correlation process because in most sections it is represented by only one site, even when sampled at 1.5 m intervals and is therefore likely to be genuinely short and thus undetectable from the seafloor spreading record. Accepting that the long normal DN1 is Epoch 9, and that thickness is approximately linearly related to time over this interval, then the best match to the time scale (that requiring the least condensing or stretching of the section) is that adopted in Fig. 2*C*. Thus the youngest of the three normal subzones DN4 within the reversed zone seems to be a previously undetected normal event within Epoch 8.

The results presented here emphasise the importance of adequate specimen demagnetisation and sampling density. Based on the previous reconnaissance level work the correlation appeared quite reasonable, but this detailed work has revised the age scale significantly. The bulk of the hominoid localities in the Khaur region fall within a narrow band associated with the U stratigraphic marked bed and a date of 8 Myr is apparently much more reasonable than the previous 9 Myr.

Even with adequate sampling density, palaeomagnetic stratigraphy in Middle Siwalik rocks is more uncertain than in rocks younger than about 5 Myr because (1) the time scale itself is less well defined and (2) the rocks have undergone prolonged diagenetic alterations (such as red pigment formation) which often obscures the original magnetisation of the rock. For these reasons, work is in progress to extend the magnetic stratigraphy pattern into younger rocks in order to increase the reliability of the correlation and the inferred ages.

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Involvement of hydrogen sulphide in the degradation of parathion in flooded acid sulphate soil

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There is increasing evidence that certain pesticides, resistant to degradation in aerobic systems, undergo fairly rapid degradation in anaerobic ecosystems such as flooded soil[1,2]. After flooding, the soil components NO_3^-, Fe^{3+}, Mn^{4+} and SO_4^{2-} are reduced almost in a thermodynamic sequence with a corresponding shift in favour of anaerobic microorganisms[3]. Although anaerobic microorganisms have been implicated directly in the degradation of some pesticides in anaerobic environments[1,2], there is circumstantial evidence for chemically catalysed reactions, involving, for example, the iron redox system[4] or reduced iron porphyrins[5]. However, the importance of this and other dominant redox reactions in the behaviour of pesticides in a flooded soil is not well understood. We report here the involvement of hydrogen sulphide, the end product of sulphate reduction, in the degradation of parathion in a flooded acid sulphate soil.

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Portions (10 g) of laterite (*p*H, 6.3; organic matter, 2.88%; S, 0.01–0.03%) and acid sulphate (*p*H, 5.2; organic matter, 5.52%; S, 0.2%; known as pokkali) soils were reduced by flooding with 10 ml of distilled water for 60 d in screw-capped glass tubes (30×120 mm, 30 ml capacity). The 60-d flooded soils were shaken with 160 μg of parathion in acetone for 30 min. The soils, flooded immediately before equilibration with parathion, served as oxidised soils. After 30 min of equilibration, the residues of parathion and its metabolites were extracted from the soils with acetone–sodium sulphate–hexane and the hexane layer was analysed by gas-liquid chromatography using a phosphorus-specific photometric detector. To measure soil reduction due to flooding, the 60-d flooded soil sample in each tube was transferred to a beaker and redox potentials were assayed immediately with a compound platinum–calomel electrode as described before[6]. The variations in soil potential in five replicates were less than 5% by this method. Redox potentials decreased from +260 to −140 mV in laterite soil and from +200 to −200 mV in acid sulphate soil after 60 d of flooding, confirming the reduction of the soil.

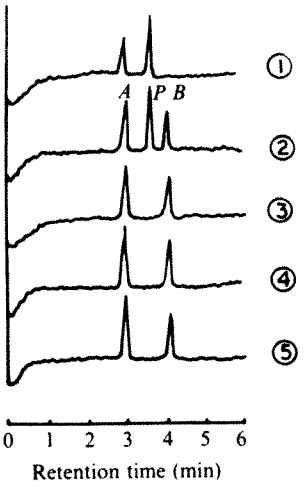


Fig. 1 Gas chromatograms of the products of reactions between: 1, parathion and prereduced laterite soil; 2, parathion and prereduced acid sulphate (pokkali) soil; 3, aminoparathion and prereduced pokkali soil; 4, aminoparathion and sodium iodide; 5, aminoparathion and hydrogen sulphide. *A*, aminoparathion; *P*, parathion; *B*, desethyl aminoparathion. The soils were prereduced by flooding for 60 d before equilibration with parathion (1 and 2) and aminoparathion (3).

After 30 min of equilibration with 60-d flooded soils, the concentration of parathion decreased to 13.8% of the original level in the acid sulphate soil and to 52.1% in the laterite soil. During the same period, 92–95% of added parathion was recovered from the oxidised soils. The gas chromatograms of the residues (Fig. 1) showed that the rapid destruction of parathion in equilibration with reduced soils led to the formation of one compound (peak *A*) in laterite soil and two compounds (peaks *A* and *B*) in acid sulphate soil. Based on chromatographic characteristics (retention time, 2.9 min; R_F, 0.35) and its positive reaction to palladium chloride spray, compound *A* was identified as aminoparathion, a major product of parathion metabolism in soils and in microbial cultures[7].

To characterise compound *B*, authentic aminoparathion was equilibrated with 60-d flooded pokkali soil for 30 min. A gas chromatogram of the residues showed the formation of a compound with the same retention time as that of compound *B* (Fig. 1), which we therefore presume was formed after aminoparathion.

We investigated whether compound *B* was a dealkylation product of aminoparathion as follows. Desethyl aminoparathion was prepared by a reaction between aminoparathion and sodium iodide, essentially as described by Miyamoto *et al.*[8]. Portions of 20 ml each of 0.05 M solutions of aminoparathion and sodium iodide in acetone were refluxed at 65±1 °C in a water bath for 3 h. This chemical reaction produced only desethyl aminoparathion, which had characteristics (retention time, 4 min; R_F, 0.47; positive reaction to palladium chloride spray) identical to those of compound *B*. Thus compound *B* was identified as desethyl aminoparathion. This is the first report of the formation of a dealkylation product in the degradation of parathion in soils. There is evidence that the degradation of related organophosphates, methyl parathion and fenitrothion, by *Bacillus subtilis* leads to the formation of their respective amino analogues and dealkylation products, desmethyl aminoparathion and desmethyl aminofenitrothion[8].

It is interesting that desethyl aminoparathion was generated from added parathion or aminoparathion only by reduced pokkali soil and not by laterite soil. Unlike laterite soil, pokkali soil is rich in organic matter and sulphate and is classified as acid sulphate soil[9]. Sulphate reduction leading to the formation of hydrogen sulphide is important in flooded soils, especially rich in organic matter and sulphate[3]. Studies in our laboratory (R. C. Ray, unpublished results) showed that after the redox potential of the flooded pokkali soil had dropped to −160 mV—a potential favourable for sulphate reduction[10]—there was significant production of hydrogen sulphide and decrease in sulphate content. In contrast, the formation of sulphide in laterite soil was negligible. These results confirmed the substantial formation of sulphide in pokkali soil when it is flooded.

To investigate the involvement of hydrogen sulphide in the dealkylation of aminoparathion, hydrogen sulphide evolved by reaction between sodium sulphide and dilute hydrochloric acid was bubbled through a solution containing 1,000 μg of aminoparathion in 2 ml of acetone and 25 ml of distilled water. The product of the reaction was extracted with acetone–sodium sulphate–hexane and analysed by gas-liquid chromatography. Only one product was detected and that had a retention time similar to that of desethyl aminoparathion and compound *B* formed by the reaction of parathion and aminoparathion with reduced pokkali soil (Fig. 1). When parathion was treated similarly with hydrogen sulphide, aminoparathion and desethyl aminoparathion were not formed. This implies that hydrogen sulphide formed readily in flooded pokkali soil was, at least in part, responsible for the formation of the dealkylation product from parathion through aminoparathion. These findings indicate that chemical degradation of pesticides mediated by the products of anaerobic decomposition of inorganic and organic soil components can be more common and widespread in anaerobic ecosystems than hitherto believed.

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Intermodal matching by human neonates

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Normal human adults judge two identical objects to have the same shape even when they are perceived through different modalities, such as touch and vision. The ontogenesis of man's capacity to recognise such intermodal matches has long been debated. One hypothesis is that humans begin life with independent sense modalities and that simultaneous tactual and visual exploration of shapes is needed to learn to correlate the separate tactual and visual sense impressions of them[1-3]. A second hypothesis is that the detection of shape invariants across different modalities is a fundamental characteristic of man's perceptual-cognitive system, available without the need for learned correlations[4-7]. Recent research has shown that 6-12-month-old infants can recognise certain tactual-visual matches[8-11]. However, such data cannot help resolve the classic theoretical debate. Infants of this age repeatedly reach out and touch objects they see, and such simultaneous bimodal exploration presumably offers ample opportunity for learning to correlate tactual and visual sense impressions. The experiments reported here show that humans can recognise intermodal matches without the benefit of months of experience in simultaneous tactual-visual exploration. We demonstrate that 29-day-old infants can recognise which of two visually perceived shapes matches one they previously explored tactually, thus supporting the second hypothesis listed above.

For our assessment of intermodal matching, we adapted a paradigm used to test infant memory[12]. We began with a brief familiarisation period during which the infant tactually explored an object. Next, the infant was shown a pair of visual shapes, only one of which matched the tactual stimulus. Visual fixation to the matching versus non-matching shape was then recorded. Three experimenters were used to ensure objectivity of the results. One experimenter selected the tactual shape and the left-right positioning of the visual shapes. This experimenter was not involved with testing the infant. A second experimenter administered the tactual stimulus. He was not informed about the left-right positioning of the visual shapes. A third experimenter observed the infant's visual fixations through a 0.64-cm peephole in the centre of the rear wall of the testing chamber. He was unaware of both the tactual shape used and the left-right positioning of the visual shapes. Corneal reflections of the test objects were visible to this scorer, but the shapes of the objects were not resolvable. He scored the infant as fixating the left object when the left reflection was visible in either of the infant's pupils, and as fixating the right object when the right reflection was visible[13]. These fixations were recorded on a Rustrack event recorder. Inter-observer agreement was high when assessed in both experiments (0.93, experiment 1; 0.98, experiment 2).

Thirty-two full-term infants ranging from 26 to 33 days old (mean 29.4 d) served as subjects in experiment 1. As infants of this age will not explore objects manually, the tactual stimuli were constructed by modifying pacifiers so that small, hard-rubber shapes could be mounted on them (Fig. 1). The matching shapes used for the visual test were constructed from dense Styrofoam and painted bright orange (diameter 6.4 cm). The experiment started with a 90-s tactual familiarisation period during which infants orally explored one of the tactual stimuli. This stimulus was then removed and the infant presented with both visual shapes for a 20-s visual test. Care was taken to ensure that the tactual stimulus was administered and removed without the infant seeing it. The shape used for tactual familiarisation, the left-right positioning of the visual objects, and the sex of the infants were counterbalanced. Thus, half the infants were tactually familiarised with the sphere and half with the sphere-with-nubs; half the infants in each of these groups were shown the familiar shape on the left and half on the right; half the infants within each of these subgroups were male and half female.

The results clearly demonstrate that infants under 1 month of age are capable of intermodal matching (Table 1). Of the 32 infants, 24 fixated the shape matching the tactual stimulus longer than the non-matching shape. These results were significantly different from chance ($P < 0.01$; binomial test). The mean per cent of total fixation time directed to the matching shape was 71.8%, as compared with the chance level of 50% ($t = 3.07$; $P < 0.01$). There were no significant differences due to sex of the infant, familiarisation object or method of feeding (breast or bottle), nor were there significant preferences for fixating the right versus left side or for fixating the sphere versus sphere-with-nubs.

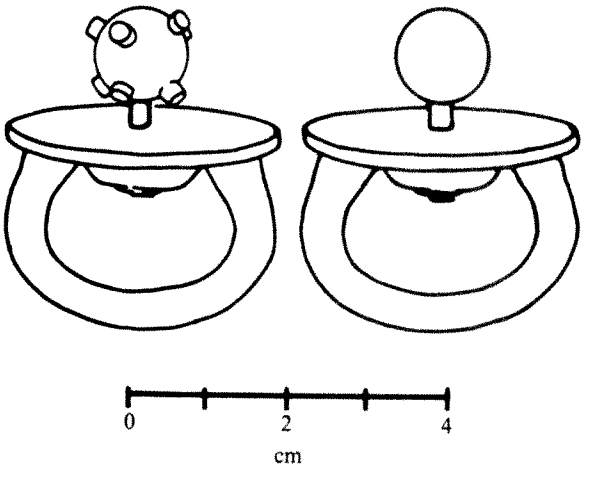


Fig. 1 The tactual objects. The tactual shapes were 1.2 cm in diameter. They were moulded from G.E. RTV-620A silicone rubber and fixed to a threaded nylon rod (6–32) that was bolted to the pacifier backing. The sphere-with-nubs was administered so that four of the nubs were orientated towards the base of the infant's mouth and four towards the roof. The nubs on the sphere were 2 mm high × 3 mm wide. The tactual stimuli were administered while the infant sat in an infant chair with his back to the visual objects. They were administered without the infant seeing them by having the experimenter hide them in his cupped hand while he was bringing the objects to and from the infant's mouth. The tactual familiarisation period started as soon as the infant began sucking on the tactual shape. Ten seconds before the end of the familiarisation period, the room lights were extinguished and the infant's seat was swivelled around so that he faced the visual objects. When the familiarisation period ended, the experimenter removed the tactual object, centred the infant's head midway between the visual objects, and only then switched on the light illuminating the visual objects. As soon as the infant made his first fixation to either object, the visual test period began, and the experimenter removed his hands from the infant. The centres of the visual objects were 28 cm apart and 32 cm from the infant's eyes. They were displayed in a three-sided black cardboard chamber 71 cm high × 112 cm wide × 52 cm deep, and each suspended from the ceiling of this chamber by a thin black rod (diameter 0.64 cm). Illumination for the visual objects was provided by an incandescent bulb directly above and behind the infant's head, yielding a luminance of approximately 0.86 log cd m^{-2} at the objects and −0.14 log cd m^{-2} at the background midway between the objects. The visual sphere-with-nubs was orientated so that four of the nubs directly faced the infant. Each nub on the visual object was 1.1 cm long and 1.2 cm in diameter and fashioned from a section of wooden doweling with the edge rounded.

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Table 1 Numbers of infants who looked longer at the object matching one they had explored tactually, compared with those who looked longer at the non-matching object

	n	Looked longer at matching shape	Looked longer at non-matching shape	P (binomial test, two-tailed)
Expt 1	32	24	8	0.01
Expt 2	32	22	10	0.05

Studies using paired-comparison paradigms usually report a preference for the novel stimulus. The present findings are not incompatible with this, as there are several important procedural differences between the present experiments and those previously reported. For example, the present experiments (1) used infants younger than typically tested with the paired-comparison technique, (2) assessed tactual–visual rather than visual–visual matching, (3) used three-dimensional forms rather than two-dimensional patterns, (4) used a 90-s tactual familiarisation period. These differences may interact to influence the direction of infant visual preference.

A second experiment checked whether different experimenters using a different sample of infants could replicate the previous effects. A new group of 32 full-term infants 27–31 days old (mean 29.4 d) served as subjects. The procedure was identical to that used in experiment 1 and the effects were replicated (Table 1). Of the 32 infants, 22 fixated the matching shape longer than the non-matching one ($P = 0.05$). The mean per cent of total fixation time directed to the matching shape was 67.1% ($t = 2.14$; $P < 0.05$).

These experiments used a successive, intermodal matching task. The tactual object was no longer in the perceptual world at the time the infants were presented with the visual test. Positive results from such a task indicate that neonates can (1) tactually discriminate between the shapes presented, (2) visually discriminate between them, (3) store some representation of the tactually perceived shape, and (4) relate a subsequent visual perception to the stored representation of the tactually perceived shape. The last point has implications for our current theories of infancy.

A basic assumption of piagetian theory[3] is that infants begin life with independent sense modalities that gradually become intercoordinated with development. Our findings, however, show that neonates are already able to detect tactual–visual correspondences, thereby demonstrating an impressive degree of intermodal unity. Thus, whatever develops in the first year of life, it is apparently not the *de novo* coordination of functionally independent sense modalities. A more general implication of the present research is that human neonates are not limited to processing bits of sense-specific information such as retinal images or tactual sensations. If neonates were restricted to registering such sensory elements they could not succeed on these intermodal matching tasks. Obviously, these initial experiments do not isolate the exact nature of the information perceived as invariant across the different modalities. However, they suggest that neonates are capable of using and storing surprisingly abstract information about objects in their world. This information must be abstract enough, at least, to allow recognition of objects across changes in size and modality of perception.

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Is there a 'consolidation' effect for monocular deprivation?

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During a time of high vulnerability early in a kitten's postnatal life, a few days of monocular vision can result in the control of most striate cortical neurones exclusively by the open eye[1–5]. Although the general consequences of this procedure have been known for some time[6], the mechanisms remain unknown. Because they develop rapidly[3,4], it has been suggested that the processes involved are similar to those of memory and learning. One aspect of this analogy is that memory is thought to be 'consolidated' from a weak to a stable form and that the process can be disrupted by the administration of drugs, electroconvulsive shock or physical trauma[7]. The connection with plasticity in the visual cortex has been suggested by studies in which kittens were first bilaterally deprived and then briefly exposed either monocularly or binocularly[3,4,8]. The effects of exposure were reported to be greatest if there was a delay between exposure and physiological study. Although this has been referred to as a 'consolidation' effect[3,4,5,8], it was a delay process rather than a process of stabilisation that was investigated. Furthermore, even though the evidence for this effect is not compelling, it has been assumed that the consequences of monocular deprivation in kittens with prior normal rearing also are consolidated, that is, they become accentuated if the unilateral occlusion is followed by a period without vision[5]. We tested directly the notion that short-term monocular deprivation effects are more extensive if, after unilateral exposure, a period is imposed before physiological study during which kittens are kept in darkness. We report here that the consolidation idea is not supported by our results. On the contrary, the post-monocular period spent in darkness actually diminished the consequences of the deprivation, allowing some functional recovery of binocular connections.

Kittens were reared normally until postnatal day 29, when susceptibility to deprivation effects is very high[1]. Vision through one eye was then blocked for 24 h using a large opaque contact lens. In one group of animals, study of cortical cells was begun immediately after the occlusion period. A second group was placed in a darkroom for 48 h before recording. Because 4-week-old kittens sleep a great deal, it was necessary to keep them awake during as much of the monocular exposure period as possible. All kittens were watched during most of their exposure sessions and they were periodically isolated from their mothers so that their attention could be maintained. The recording sessions were arranged so that cats were studied in pairs, one by each of us in separate laboratories. Findings were not compared until the studies were completed.

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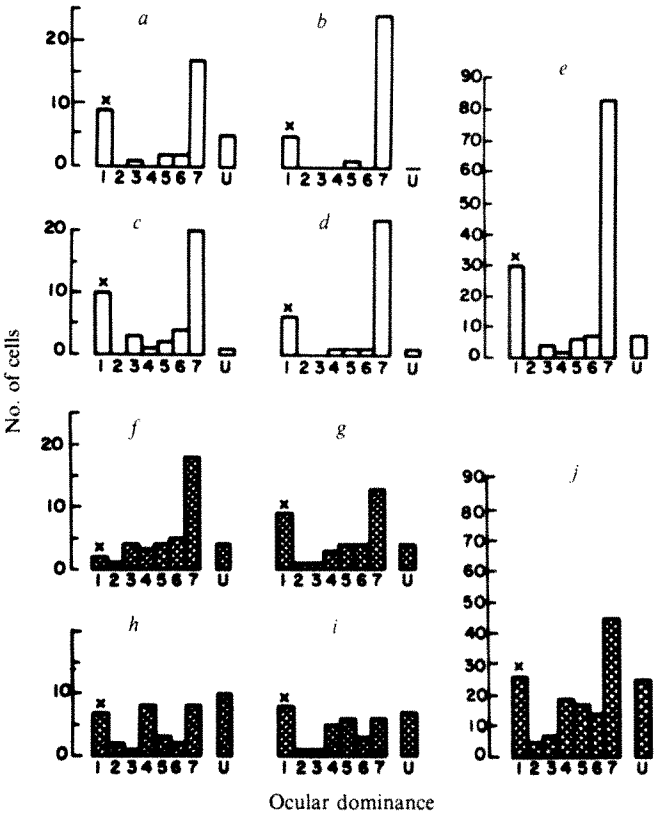


Fig. 1 Ocular dominance histograms are shown for experimental animals. The numbers on the abscissae represent subjectively determined classifications of the degree to which the two eyes influence a given cortical cell. Binocular neurones are designated by groups 2–6 and monocular cells by groups 1 and 7. Groups 1–3 (or 5–7) represent cells dominated by the eye contralateral (or ipsilateral, respectively) to the hemisphere from which electrode recordings were made. Visually unresponsive cells are indicated by the category 'U'. After 4 weeks of normal postnatal rearing, eight kittens were unilaterally occluded with large opaque contact lenses worn in their left eyes for 24 h. Half were studied physiologically immediately after occlusion (*a–d*) and the other half spent 48 h in darkness before recording (*f–i*). Summary histograms are shown for each group in *e* and *j*. × Indicates the occluded eye, which, in all cases, was contralateral to the hemisphere that contained the electrode.

Standard physiological recording techniques were used[9,10]. Halothane was used while a vein was cannulated, and anaesthesia was continued with Brevital while a tracheal tube was positioned and an electrode placed. Animals were then paralysed and artificially respirated; CO_2, electroencephalogram, electrocardiogram and temperature were monitored. Spikes isolated from individual neurones by means of tungsten-in-glass microelectrodes were amplified and displayed. As a conservative procedure, electrodes were always placed in the hemisphere contralateral to the deprived eye because, in the normal cat, the contralateral eye tends to predominate[9].

Receptive fields were determined for each responsive unit. Optimal stimuli were found for each eye and cells were classified into types using standard criteria[1,2,9,10]. We paid particular attention to subjectively determined estimates of absolute response strength and to the relative strength of responses elicited through each eye (ocular dominance). Response strength classifications used were: unresponsive, weak, fair, good and vigorous. Relative strength was categorised into seven ocular dominance groups, as defined in Fig. 1 legend.

In normal cats and kittens, most cells in the visual cortex are binocular[1,9]. Ten days of monocular occlusion in a 4-week-old kitten results in the open eye gaining control over nearly all cortical cells[2]. One day of this procedure is sufficient to produce a marked effect, as shown in Fig. 1. Ocular dominance histograms *a–d* are for kittens studied immediately after 24 h of monocular deprivation. A summary of these results is given in *e*. Two marked effects are evident. First, very few binocular cells were found; second, considerably fewer cells were controlled by the deprived eye in all cases (27% of the total are contralateral, that is, fall into groups 1–3). Most cells were highly responsive to optimal stimuli and, as shown in the histograms, very few were visually unresponsive. Data from the animals placed in darkness for 48 h following 24 h of monocular exposure are shown in Fig. 1*f–i*, with a summary in *j*. Once again, there is a clear reduction of binocularity, but it is noticeably less than in the first group (binocular cells constitute 14% in the first group *e* and 47% in the second *j*). The findings for the group which was studied after a delay also indicate a predominance of ipsilateral (groups 5–7) cells (64%). Although this is less than the proportion observed in the group recorded immediately after monocular exposure (73%), the difference is not statistically significant (χ^2 test). The summary histograms (Fig. 1*e*, *j*) show one other prominent difference between the two groups. Visually unresponsive cells comprise only 5% (7 cells) of the total in the first group *e*, but 16% (25 cells) in the second group. In addition to the higher proportion of unresponsive cells, the imposition of 48 h of darkness (second group) seemed, in general, to cause a noticeably reduced strength of visual responsiveness even when cells were driven through the eye that had not been deprived. More units habituated, had bursting responses, exhibited high spontaneous activity and seemed generally variable than in the other group. As a consequence, more cells were classified as weak or fair. Apparently, the relatively short period with no visual input was sufficient to cause some degenerative changes in cortical connections.

The results presented in Fig. 1 clearly show that the period spent in darkness following 24 h of monocular exposure does not produce a consolidation of the unilateral deprivation effect. On the contrary, a substantial increase occurs in the proportion of cells that can be driven through both eyes. However, the conclusion that a consolidation period does not accentuate the effects of monocular deprivation is limited to the time courses we chose for unilateral exposure and for the post-exposure period. It is possible that a consolidation effect occurs, but over a different time course. The loss of binocularity for the animals recorded after 24 h of unilateral deprivation was so marked that a greater effect would be difficult to discern.

We decided, therefore, to examine two other groups of kittens with a shortened time course of monocular exposure. In the first, animals were unilaterally occluded for 8 h on postnatal day 29 and then studied physiologically. The second group was similarly exposed monocularly, but recording was delayed for an additional 8 h during which the kittens were kept in darkness. Because of the very brief time, animals were exposed in a slowly rotating clear chamber to ensure that they were alert during most of the period. Once again, analysis of a sample of cortical cells showed that binocularity was severely reduced for both groups of cats, although not as much as for those shown in Fig. 1. In three cats recorded immediately after monocular deprivation, 36% of all cells were binocular, compared with 51% in three others recorded after 8 h of darkness. In addition, the ipsilateral eye (non-deprived) predominated in both cases, but controlled 70% of the sample from the first group and 58% from the second which had an 8-h interval between exposure and physiological study. As in the case of 24-h monocular deprivation, these findings do not support the notion of consolidation. The degree of binocularity is once again actually higher when a period intervenes between exposure and recording. Therefore, we conclude that for this condition also, there is no consolidation effect.

We have shown that periods as short as 8 h of monocular deprivation can cause functional disruption of binocular cortical connections. This finding is distinct from that of previous studies in which short periods of monocular exposure were preceded by binocular deprivation[4,11]. In that case, the physiological effects

observed reflect a unilateral recovery of visual function and the mechanisms could be different from those of monocular deprivation. The fact that the effects we have found are so much greater than those previously reported[5] may be due in part to the procedures we used to ensure maximum exposure during the monocular rearing periods. However, the major difference is probably attributable to use, in the previous study, of a post-monocular period of binocular deprivation.

In the conditions of our study, we conclude that the effects of short-term monocular deprivation do not become consolidated during a post-exposure period spent in darkness. The inactivation of pathways from a deprived eye must require some time, but it must be much shorter than the period of deprivation required to produce an effect detectable by our methods. We have not tested 'consolidation' in the original sense[12,13], because our study did not attempt to look at the issue of conversion of a deprivation effect from an unstable to a stable form. To do this, one might administer, for example, electroconvulsive shock or a protein synthesis inhibitor like puromycin, to kittens immediately after a period of monocular exposure to see if these agents interfered with the effects of the deprivation.

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The analysis of sound by the sprat ear

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In the acoustico-lateralis systems of vertebrates the individual hair cells are usually polarised in their responses to displacements of the liquid in which they lie, and are often arranged in back-to-back pairs or groups with different polarities. A simple example to investigate, mechanically as well as electrically, is the utriculus of the sprat (*Clupea sprattus L.*). The acoustico-lateralis system of the sprat and other clupeids has two partly gas-filled bony bullae which transform pressure changes into liquid displacements capable of stimulating the sense organs of the ear and lateral line[1–3]. With its related structures the utriculus is a very sensitive sound pressure detector which has one population of receptors that respond to the compressions and another that respond to the decompressions of a sound wave. We now give additional evidence that this type of organisation is unlike that of the mammalian cochlea in being specialised more for the detection of phase/time relationships than for frequency analysis.

Popper and Platt[4] have described the distribution and orientation of hair cells in the three maculae of the herring utriculus[5]. In each macula the hair cells have their kinocilia on the side closest to a line running down the middle of the long axis. One of us (J.A.B.G.), with A. C. G. Best, has independently made similar observations on the sprat utriculus. This is like that of the herring except that the hair cells of the posterior macula are orientated along, rather than across, its length. The anterior and middle maculae are suspended along their long edges (Fig. 1). Each sprat utriculus has about 13,000 hair cells, about 60% on the middle macula and slightly more than 20% on the anterior macula.

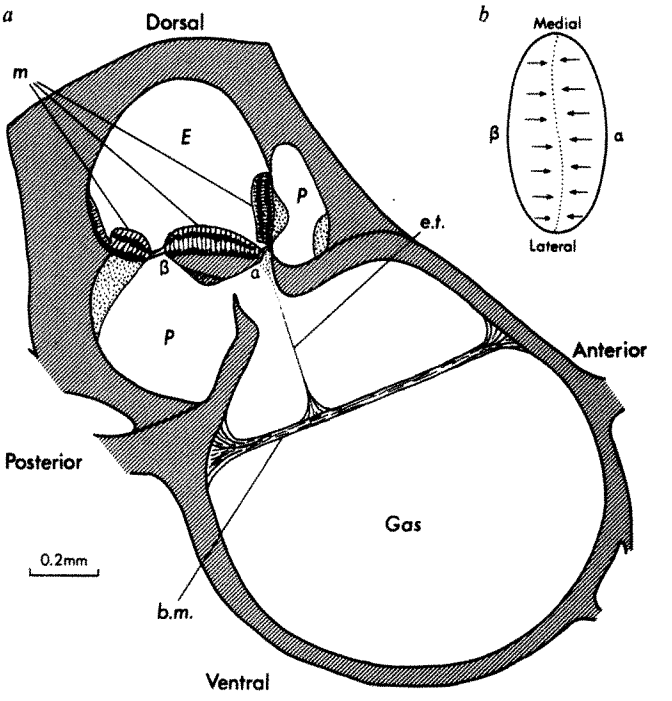


Fig. 1 *a*, Sagittal section through part of the auditory system of the sprat. Pressure vibrations cause vibrations of the bulla membrane (b.m.) which set up vibrations of the utricular maculae (m). The long axis of the middle macula is at right angles to the line $\alpha\beta$. The hatched areas are tough collagenous or calcified tissue. Nerves are stippled. The maculae are shown as cells but the 'hairs', cupulae and the utricular otolith are not shown. A thin elastic thread (e.t.) connects the bulla membrane to the base of the utriculus (out of the plane of this drawing). It seems possible that this thread is associated with the detection of slow, large pressure changes. E is endolymph, P perilymph. Displacements of liquid are possible because the system communicates with the lateral line. *b*, Plan view from above of the middle macula of the utriculus. It is divided into two areas in which the hair cells are orientated in opposite directions. The arrows point to the sides of the hair cells on which the kinocilia lie.

As an electrode is introduced into the utricular endolymph a positive d.c. potential step of 10–14 mV is observed. On stimulation with sinusoidal pressure waves microphonic potentials of double frequency[6,7] are recorded. The potentials consist of two sets of negative peaks, one associated with compressions, the other with decompressions (Fig. 2*a*). Because in fish, hair cells are preferentially excited when their ciliary processes are displaced towards their kinocilia[8], it seems almost certain that the two sets of peaks correspond to the excitation of the two major populations of oppositely orientated hair cells in the two larger maculae. These responses do not change significantly in shape over a frequency band of at least 5–880 Hz. The two sets of peaks are similar in the way they change when the electrode is moved in and out of the endolymph, in the way they decline with anoxia and in the time intervals between the peaks of each set and the corresponding compressions or decompressions. The recorded responses to a pressure of 1 N m^{-2} are of a few tens of microvolts and attain about half a millivolt at pressures around 100 N m^{-2}. We shall call the responses to compression c-responses (from c-receptors) and the responses to decompression d-responses (from d-receptors). The pressure required to give a particular response of either group changed very little between about 40 and 700 Hz. Below about 20 Hz sensitivity declined sharply.

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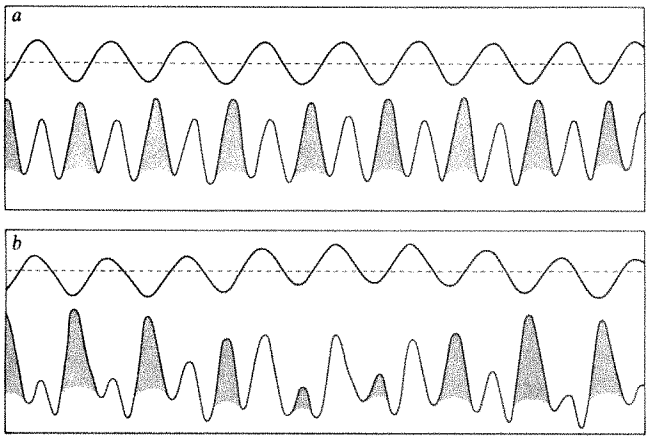


Fig. 2 *a*, Upper trace: stimulus, a sinusoidal pressure wave of 200 Hz; mean pressure shown by dashed line, compressions upwards; peak to peak pressure 12.5 N m^{-2}. Lower trace: potentials in the endolymph of the utriculus; *d*-responses are stippled. *b*, Like *a* but the stimulus pressure obtained by mixing frequencies of 200 Hz (as in *a*) and 30 Hz.

Two frequencies of pressure stimulation can be combined (see ref. 7) as, for example, in Fig. 2*b*, where 200 and 30 Hz waves are mixed. The d-responses to the 200-Hz component (stippled) are larger during the decompression phase of the slower wave and smaller during the compression phase. The c-responses behave in the reverse way. In Fig. 2 the envelopes enclosing both the c- and d-responses correspond to the lower frequency. The two frequencies and their phase relationship are much clearer in the responses of the receptors than in the pressure record. The responses of the two different receptor populations result from pressure excursions either above or below the mean pressure and their timing can be predicted from the form of the pressure wave. The sizes of the responses increase with the extent of the compression or decompression, but this relationship is not the same as that of the d-response to simple sine wave stimulation, for the slope of the log response/log pressure curve is much steeper.

The phenomena described above also produce potential patterns which are very sensitive to phase between harmonics. Figure 3 shows utricular responses to changes of phase between a pressure wave of 62.5 Hz and its second harmonic.

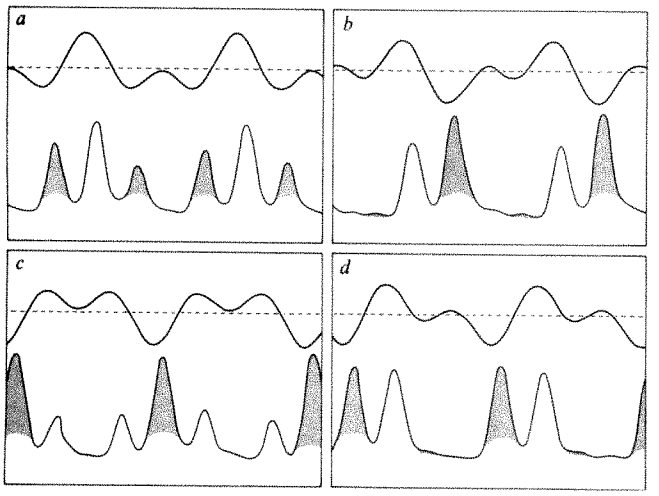


Fig. 3 To show that phase changes greatly affect the pattern of the responses given by the utriculus. *a*, Upper trace: stimulus pressures obtained by mixing frequencies of 125 Hz and 62.5 Hz of approximately equal amplitudes (~15 N m^{-2}); mean pressure shown by dashed line, compression upwards. Lower trace: potentials in the endolymph of the utriculus; the d-responses are stippled. *b*, *c*, *d*, Like *a* but with the higher frequency delayed by 2, 4 and 6 ms, respectively (respective delays in phase 90°, 180° and 270°).

These results give information on the representation of amplitude and timing in the activity of the utriculus. As a considerable proportion of the 13,000 cells is likely to be available, it seems probable that amplitude can be coded accurately and quickly by the number of active units in the population of nerve fibres. The intervals between compressions or decompressions or between a compression and a decompression are clearly represented even in a complex wave by the activities of the two populations of receptors. The system should allow interactions which diminish noise and blurring. The striking changes in the relative amplitudes of the c- and d-responses which immediately follow changes in the pressure wave strongly suggest that the system is organised to facilitate the detection of time/phase differences and provide rapid answers to rapid events, that is, to noises or transients given, for example, by a sharp change of direction of a neighbouring fish[9]. It also provides a 'standard' based on pressure against which the activity of the various neuromasts of the lateral line can be compared, thus enabling estimates of distance and direction to be made[3,10].

As so much is known about hearing in mammals, it is interesting to contrast the behaviour of the clupeid utriculus with that of the mammalian cochlea. Microphonics recorded from the latter are very different from those described above; there is no frequency doubling and the response to a sinusoidal stimulus is an almost perfectly sinusoidal wave of the same frequency (see for example, ref. 11).

Helmholtz and others have shown that, with trivial exceptions, man is insensitive to phase relationships of tones[12,13]. Results like those of Figs 2 and 3 support the view that this may not be true for animals in which the receptors are organised in the same general way as the sprat utriculus.

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Met- and Leu-enkephalin immunoreactivity in separate neurones

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A pair of pentapeptides, Met- and Leu-enkephalin were recently isolated from brain tissue[1,2]. The two peptides seem to represent endogenous opiate receptor ligands and have by immunocytochemical and radioimmunoassay studies been shown to occur in an extensive system of cerebral and peripheral nerves[3–8]. The relative proportions between Met- and Leu-enkephalin varies between different brain regions and also between different species, suggesting the existence of separate populations of Met- and Leu-enkephalin nerves[9–12]. Until now, however, immunocytochemistry has given no support for this notion. We report here evidence of separate populations of Met- and Leu-enkephalin nerves.

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Table 1 Properties of enkephalin antisera determined by radioimmunoassay

Serum	Dilution	IC_{50} (pmol of peptide) Met-enk	Leu-enk	β-endorphin
anti-Met-enk M12	1:250	40	4,500	>25,000
anti-Met-enk M14	1:250	25	2,000	>25,000
anti-Leu-enk L2	1:500	500	3	>25,000

Radioimmunoassays were performed as previously described[21], using a dilution of serum which bound 30–40% of ^{3}H-enkephalin in the absence of unlabelled peptide. Results of competitive binding experiments are expressed as the IC_{50} values of the amounts of peptides required to displace 50% of bound ^{3}H-enkephalin in the radioimmunoassay. The incubation volume was 0.25 ml. The data were obtained from typical experiments performed in triplicate and repeated at least twice.

Met- and Leu-enkephalin differ only in that their COOH-terminal residue is either methionine or leucine. Furthermore, Met-enkephalin is identical to the NH_2-terminal pentapeptide of the long-chain opioid peptide, β-endorphin. So far, however, there is no evidence for a biosynthetic relationship between these two peptides. Furthermore, immunocytochemistry has demonstrated that enkephalin-immunoreactive nerves show a distribution distinct from that of nerves containing β-endorphin, β-lipotropin (β-LPH) and adrenocorticotropic hormone (ACTH)[6,13,14]. The similarities in amino acid sequences may, however, cause significant problems of cross-reactivity between different opioid peptides. In several previous studies, radioimmunoassay data have been obtained indicating only small degrees of cross-reactivity between the two enkephalins[11,12]. Such data cannot, however, be taken as proof for immunocytochemical specificity[15,16]. Thus, of numerous reports on enkephalin immunocytochemistry[3–8,17] to our knowledge, only one, dealing with Met-enkephalin localisation[5], seems to have fully documented lack of cross-reactivity.

We have examined a number of antisera to Met- and Leu-enkephalin in immunocytochemical models and have obtained evidence for considerable cross-reactivity. With selective absorptions as well as by oxidative destruction of the methionine residue of Met-enkephalin we have been able to develop methods, selectively demonstrating either Met- or Leu-enkephalin. When applied to tissue sections, these selective techniques reveal that the two enkephalins are localised in two distinct systems of nerves.

Three antisera were used (Table 1). In radioimmunassay both Met-enkephalin antisera, M12 and M14, displayed approximately 100-fold greater selectivity for Met-enkephalin than Leu-enkephalin. The Leu-enkephalin antiserum L2 had over 150 times more affinity for Leu-enkephalin than Met-enkephalin. None of the three antisera exhibited any cross-reactivity with concentrations of β-endorphin as high as 10^5 pmol ml^{-1}.

Cytochemical models consisted of Sepharose 4B beads, to which was coupled either synthetic Met-enkephalin, Leu-enkephalin or β-endorphin. Treated beads were attached to glass slides by the technique of Streefkerk *et al.*[18] and subjected to immunocytochemical staining (peroxidase–antiperoxidase, PAP procedure). L2, M12 and M14 antisera were all found to react with beads to which Met- and Leu-enkephalin was coupled, whereas, at all concentrations tested (1:200–1:40,000), they failed to react with the β-endorphin beads. In accord with this observation, pre-adsorptions of the three antisera with β-, α-, and γ-endorphin, ACTH or other unrelated peptides (see Fig. 1) failed to affect staining of the Met- and Leu-enkephalin beads. Pre-adsorption of all three antisera with Met-enkephalin (2–100 μg ml^{-1}) resulted in complete inactivation of staining of both Met- and Leu-enkephalin beads. In contrast, pre-adsorption of antisera M12 and M14 with

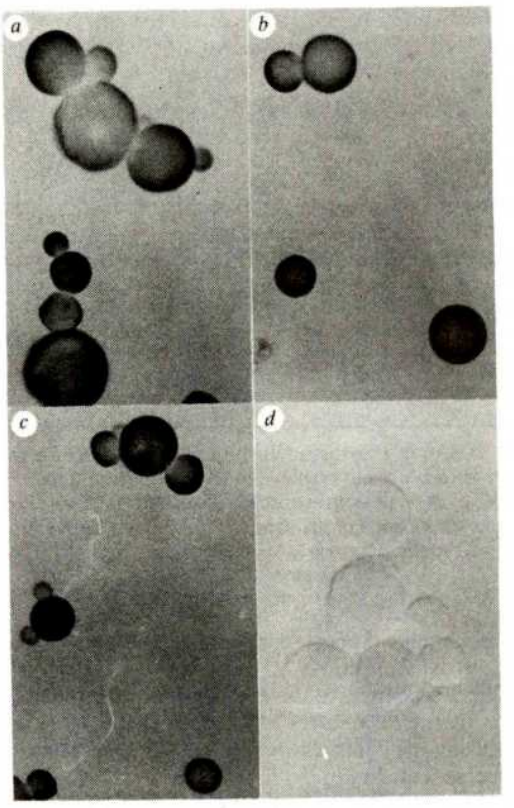


Fig. 1 Sepharose beads to which Leu-enkephalin (*a*, *b*) or Met-enkephalin (*c*, *d*) has been coupled. The beads shown in *a* and *c* have been reacted with Leu-enkephalin antiserum (L2; PAP technique) without pretreatment, whereas the beads in *b* and *d* have been pre-oxidised with acidic permanganate before staining with antiserum L2. Note that this antiserum reacts as readily with Met- as with Leu-enkephalin (*a*, *c*) and that oxidation with acidic permanganate eliminates staining of Met- (*d*) but not of Leu-enkephalin (*b*). Thus, following this type of pre-oxidation, antiserum L2 is fully specific for Leu-enkephalin. Similar model staining experiments confirmed that Leu-enkephalin-adsorbed antisera M12 and M14 were fully specific for Met-enkephalin. Cytochemical models were prepared by coupling synthetic Met-enkephalin, Leu-enkephalin or β-endorphin (Bioproducts Peptide and Peninsula Labs) to CnBr-activated Sepharose 4B beads (Pharmacia). In general, 1 mg of peptide was coupled to 2 ml swollen beads according to the manufacturers manual. Prepared beads were mounted as monolayers on glass slides according to Streefkerk *et al.*[18] and subjected to various treatments before immunocytochemical staining. Such treatments included exposure to solutions used for preparing the tissue material (compare with Fig. 2) and excluded deleterious influences of these procedures on the Met- or Leu-enkephalin immunoreactivity. In addition, the beads were treated with solutions of potassium permanganate in dilute sulphuric acid. The best formula agreed with that for antibody elution suggested by Tramu *et al.*[19], and consisted of 0.15 M $KMnO_4$ and 0.02 M H_2SO_4. Treatment of beads (or cryostat sections) with this solution for 2 min was sufficient to completely block Met-enkephalin immunoreactivity, whereas even a 10-min exposure left Leu-enkephalin immunoreactivity unaffected (as tested by antiserum L2). Oxidised and non-oxidised beads were allowed to react with either unpretreated, Leu-enkephalin-pretreated or Met-enkephalin-pretreated antisera to Met-enkephalin (M12, M14) or Leu-enkephalin (L2). Antisera were produced in rabbits by immunisation with the enkephalins coupled by carbodiimide and glutaraldehyde to keyhole limpet haemocyanin, as previously described[21]. The site of antigen–antibody reaction was revealed by either PAP or immunofluorescence[22]. Controls for immunocytochemical specificity were made by pretreating antisera with varying amounts of Leu- or Met-enkephalin (2–100 μg ml diluted antiserum) as well as with excess amounts of α-, β- and γ-endorphins, ACTH (Ferring) and synthetic human gastrin I (ICI). Of these peptides, only the enkephalins affected staining and additional controls including conventional staining controls[22], gave negative results.

Leu-enkephalin (5–100 μg ml^{-1}) failed to abolish staining of the Met-enkephalin beads, whereas it abolished staining of the Leu-enkephalin beads. On the basis of these results we conclude that the Met-enkephalin antisera M12 and M14 contain two antibody populations: one specific for Met-enkephalin and one reacting with both Met- and Leu-enkephalin. The latter antibody population could be inactivated by pretreating the antisera with as little as 5 μg of Leu-enkephalin, but in all subsequent studies this antiserum was used routinely with 100 μg Leu-enkephalin added per ml of a 1 : 1,600 dilution. In these conditions, both antiserum M12 and M14 are fully specific for Met-enkephalin.

We noted that even mild oxidative treatment (by acidic permanganate or cyanogen bromide) completely abolished immunoreactivity of the Met-enkephalin beads, whereas that of Leu-enkephalin beads was left unaffected. This effect can be explained by destruction of the methionine residue of Met-enkephalin and has previously been exploited in bioactivity studies[9,10]. Thus, following such oxidative treatment our cross-reactive antiserum L2 produced strong staining of Leu-enkephalin beads but left Met-enkephalin beads unstained (Fig. 1).

On the basis of such model experiments we examined the tissue distribution of Met-enkephalin by using Leu-enkephalin-absorbed antiserum M14 or M12, and of Leu-enkephalin by treating tissue sections with oxidising agents (usually acidic permanganate solutions), before staining with Leu-enkephalin antiserum L2. Immunocytochemical specificity was proved by adsorbing antisera against the enkephalins, endorphins, ACTH and other peptides (compare Fig. 1). As precursors to the enkephalins have not yet been identified it is impossible to assess whether our antisera would also react with such molecules. Note, however, that although β-endorphin contains the entire sequence of Met-enkephalin, none of the antisera used reacts with this long-chain opioid peptide. Even if our enkephalin antisera did react with enkephalin precursors, however, our results make it reasonable to assume that oxidation plus staining with antiserum L2 demonstrates only Leu-enkephalin-related molecules and that Leu-enkephalin-pre-adsorbed antiserum M12 and M14 react only with Met-enkephalin-related molecules.

Both techniques demonstrated numerous immunoreactive nerves in both the gastrointestinal tract of cats and guinea pigs and in brains of guinea pigs. In most regions studied, the number of Met-enkephalin nerves exceeded that of Leu-enkephalin nerves. The two types of nerves showed roughly the same distribution, which agreed with previous data on the distribution of enkephalin immunoreactivity in brain and gut[3–8,17]. In the cat gastrointestinal tract, occasional ganglia of the myenteric plexus seemed to contain predominantly Leu- or Met-enkephalin nerves (Fig. 2). Most areas examined, including the myenteric plexus showed, however, great parallelism in the distribution of the two types of nerves. To prove that the two peptides did occur in distinct populations of nerves, double-staining immunocytochemistry was carried out. Met-enkephalin immunoreactivity was first demonstrated by Leu-enkephalin-pre-adsorbed antiserum M12 or M14 with the peroxidase–antiperoxidase (PAP) procedure. Following demonstration of peroxidase activity with 3,3′-diaminobenzidine, which produced a brown reaction product, antibodies were eluted from the sections by the method of Tramu *et al.*[19]. This technique uses an acidic permanganate solution, which also destroys Met-enkephalin immunoreactivity. Following successful elution and simultaneous destruction of Met-enkephalin immunoreactivity, the sections were restained with antiserum L2, now able to react only with Leu-enkephalin present in the sections. The PAP technique was used again, but peroxidase activity was now demonstrated with 4-Cl-1-naphtol, yielding a blue reaction product[19]. Thus Met-enkephalin-containing nerves are stained brown and Leu-enkephalin-containing nerves are stained blue.

Alternatively, the site of reaction between the L2 antiserum and the tissue was shown by indirect immunofluorescence. In this case, brown-stained Met-enkephalin and immunofluorescent Leu-enkephalin nerves were visualised (Fig. 3). The success of the antibody elution was verified by the fact that the Met-enkephalin nerves did not restain in the second step of the double-staining procedures as well as by controls involving use of antisera pre-adsorbed with either Met- or Leu-enkephalin in both steps of the procedures and, finally, also by the substitution of the enkephalin antisera by unrelated immune sera. All these controls confirmed the specificity of the immunolocalisations and also documented the completeness of the antibody elution technique as well as its ability to destroy Met-enkephalin reactivity to antiserum L2. The double staining techniques were successful in demonstrating distinct populations of Met- and Leu-enkephalin nerves in the nucleus caudatus-putamen and globus pallidus of the guinea pig brain and in the gastrointestinal tract. Interestingly, both types of nerves showed a very similar distribution, with Leu-enkephalin nerves being about one-quarter to one-fifth as numerous as Met-enkephalin fibres. A full description of the extent and distribution of the two types of nerves will be published elsewhere.

Our data demonstrate that Leu- and Met-enkephalin occur in two distinct populations of nerves. The occurrence of two distinct types of enkephalin nerves is of considerable interest, partly because both types seem to show at least partially overlapping distributions. This distribution also agrees with that of

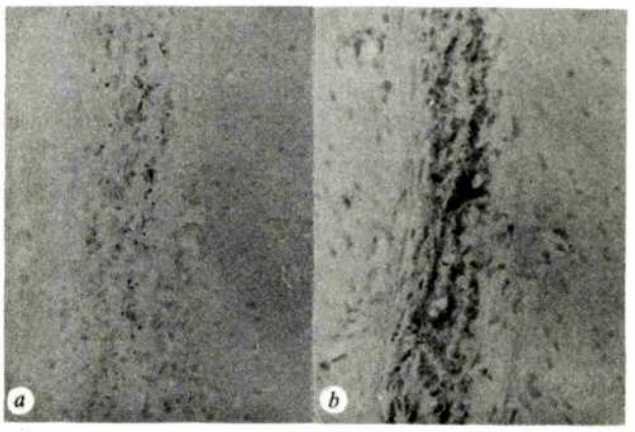


Fig. 2 Adjacent sections showing Auerbach's plexus of cat ileum. *a*, This section has been stained with Leu-enkephalin-adsorbed antiserum M14 (PAP technique), whereas *b* has been pre-oxidised with acidic permanganate and then stained with antiserum L2 (compare with Fig. 1). Thus, in *a* Met-enkephalin immunoreactive nerves and in *b* Leu-enkephalin immunoreactive nerves are shown. Note that in this particular ganglion the Leu-enkephalin nerves are more numerous than the Met-enkephalin nerves and that also a Leu-enkephalin immunoreactive cell body can be seen. Similar experiments, involving staining of the same section for the two types of immunoreactivity in contrasting colours (compare with Fig. 3), showed that the Leu- and Met-enkephalin immunoreactivity resided in separate nerve populations. Guinea pigs ($n = 10$) and cats ($n = 5$) were anaesthesised with diethyl ether or mebumal chloralose, respectively, and perfused via the heart with cold (4 °C) saline followed by a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer *p*H 7.3. Specimens from the gastrointestinal tract of both species as well as frontal brain slices from guinea pigs were postfixed in the paraformaldehyde solution for 15–30 min, soaked in 20% sucrose solution in the phosphate buffer overnight and frozen on cryostat chucks in melting Freon-22. Cryostat sections, cut at 3–10 μm, were subjected to immunocytochemical staining for Met- and Leu-enkephalin immunoreactivity, as described in Fig. 1 legend. Controls were also identical. Occasionally, sections stained for Met-enkephalin immunoreactivity were submitted to antibody elution according to Tramu *et al.*[19] and poststained with a cross-reacting Leu-enkephalin antiserum (L2). This elution technique is identical with that used for destroying Met-enkephalin immunoreactivity. Met- and Leu-enkephalin nerves could be demonstrated in contrasting colours, using the PAP technique. The efficiency of antibody elution was shown by the fact that separate nerves were stained, and by the controls, suggested by Tramu *et al.*[19].

brain opiate receptors[4]. As previously pointed out, nerves containing other putative ligands for opiate receptors, like β-endorphin and ACTH, show only a partially overlapping distribution with enkephalin nerves[6,13,14]. These data should be viewed in the light of the previously suggested existence of separate types of opiate receptors[20] and should initiate studies of differential release of the two endogenous opiate ligands.

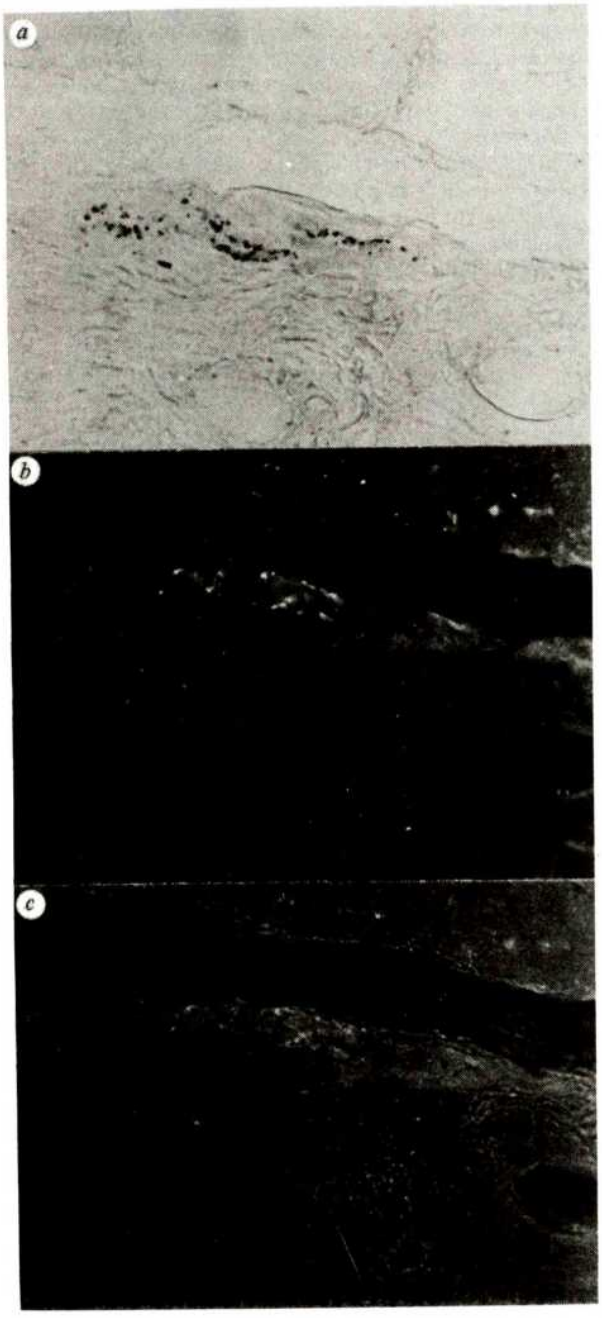


Fig. 3 Section of submucous (Meissner's) ganglion of cat small intestine. The section was first stained with Leu-enkephalin-adsorbed antiserum M14 using the PAP procedure (top) resulting in dark-stained Met-enkephalin nerves. Subsequently, antibodies were eluted and Met-enkephalin immunoreactivity destroyed by acidic permanganate treatment, whereafter the section was restained with antiserum L2, now able to show only Leu-enkephalin immunoreactivity (compare with text and Fig. 2). The site of reaction with the L2 antiserum was revealed by indirect immunofluorescence, using fluorescein isothiocyanate-labelled goat antirabbit IgG (centre). Bottom, the same section is viewed in combined blue light (490 nm) and in ordinary light, showing that the black-stained Met-enkephalin nerves are distinct from the immunofluorescent Leu-enkephalin nerves. ×370.

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A novel analgesic dipeptide from bovine brain is a possible Met-enkephalin releaser

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It is generally accepted that morphine exerts its analgesic effect by binding to specific opiate receptors in the brain and spinal cord[1]. Since Hughes *et al.*[2] isolated and identified two endogenous pentapeptides, Met- and Leu-enkephalin, from the brain and found that they acted as agonists at opiate receptors, α-, β- and γ-endorphins, larger peptides than enkephalins and having morphine-like activity, have been identified in either the brain or pituitary of various species[1]. Several studies have demonstrated that enkephalins possess analgesic properties and that they are distributed in the pain-mediated pathways in the central nervous system. These findings suggest that enkephalins are important neurotransmitters or neuromodulators regulating pain transmission. We now report the isolation of a novel substance which has a Met-enkephalin releasing action. Our findings suggest the possibility of a regulating mechanism for the release of endogenous opioid peptides, especially Met-enkephalin.

During purification, bovine brain extracts were injected intracisternally into unanaesthetised mice, using a J-shaped needle[3], and analgesia was evaluated by the tail-pinch test using an artery clip (2 mm wide) with a constant pressure of 200 g (ref. 4). All the materials tested were dissolved in distilled water and administered into the cisterna magna, in a volume of 10 μl. Eight mice were used for each experiment. The specificity of morphine-like activity was confirmed using the narcotic antagonist naloxone.

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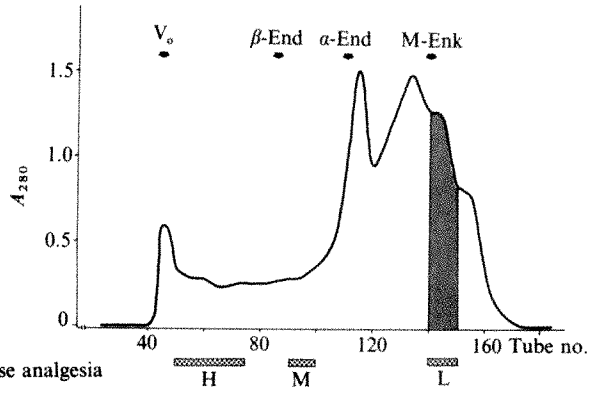


Fig. 1 Gel filtration chromatography of 3 g of 0.5 M acetic acid extract on a Sephadex G-50 column (90 × 5 cm). Elution was carried out with 0.5 M acetic acid, the rate of flow was 1 ml min^{-1}, and 15-ml fractions were collected. The elution pattern was monitored by UV absorbance at 280 nm. The analgesic activities were found in three fractions, H, M and L, indicated by the horizontal bars. The approximate elution positions of some endorphins are indicated by the arrows. V_0, void volume; β-End, β-endorphin; α-End, α-endorphin; M-Enk, Met-enkephalin.

Isolation of the substance was as follows. Fresh frozen bovine brains minus cerebella were crushed and homogenised in 5 volumes of acetone with a Polytron homogeniser at −10 °C. The homogenate was filtered and the acetone powder obtained was stirred for 12 h with 10 volumes of 0.5 M acetic acid at 4 °C. After centrifugation, the supernatant was lyophilised, redissolved in a small volume of 0.5 M acetic acid and applied to a Sephadex G-50 column. As shown in Fig. 1, analgesic activities measured as described above were found in three fractions, designated H (molecular weight ~ 10,000), M (MW ~ 3,000) and L (MW ~ 1,000). As the naloxone-induced reversibility of fraction L was more clear-cut than that of the other two fractions, this fraction was run in a Dowex 50W-X2 column to separate the peptides further. The column was eluted with a linear *p*H gradient of 0.2 M pyridine–acetate buffer, *p*H 3.1, and 1 M pyridine–acetate buffer, *p*H 7.0, and successively with 0.01 M NH_4OH, 1 M NH_4OH and 2 M NaOH. Analgesic activities were found in three fractions, L1, L2 and L3. The peptides of fraction L3 were found to be basic, as they could be eluted with 1 M NH_4OH. Fraction L3, which consisted of three peaks, could be further broken down into three fractions, L3a, L3b and L3c (see Fig. 2). In these five fractions (L1, L2, L3a–c), the most potent analgesic activity was found in fraction L3b, which showed a 38-fold enrichment in analgesic activity compared with the original L fraction. Fraction L3b was then applied to a BioGel P-2 column (Fig. 3), and the L3b′ fraction obtained showed an analgesic activity 1.5-fold greater than that of fraction L3b. The analgesic effect of intracisternally injected L3b′ peptide (12 μg per mouse) was maximal (about 55%) within 5 min, terminated 30 min after administration and was completely suppressed by pretreatment with naloxone (0.5 mg per kg, subcutaneously (s.c.)). The elution position of fraction L3b′ did not correspond with the positions of α-endorphin or glycine, and showed that this substance had a relatively small MW. With high-voltage paper electrophoresis (HVPE) at 33 V cm^{-1} for 90 min at *p*H 3.6 (pyridine/acetic acid/H_2O; 1 : 10 : 289) fraction L3b′ showed a single spot with ninhydrin reagent. The relative mobility value of peptide L3b′ was 0.75 using arginine as a standard, and its amino acid composition, after hydrolysis with 6 M HCl at 110°C for 18 h, was tyrosine 0.90 and arginine 1.00. Tryptophan was not detected with Ehrlich reagent on TLC of peptide L3b′. Moreover, dansylation and HCl hydrolysis of the peptide resulted in a single spot on the polyamine sheet corresponding to *bis*-tyrosine, and thus indicating that the single N-terminal amino acid was indeed tyrosine. In view of these results and the elution position on the BioGel P-2 column, tyrosyl-arginine is proposed as the amino acid sequence of peptide L3b′.

In comparative studies, synthetic dipeptide Tyr-Arg and the endogenous peptide L3b′ had an identical R_F value (R_F 0.18) on TLC (*n*-butanol/pyridine/acetic acid/H_2O; 4 : 1 : 1 : 2), showed only a single spot on co-chromatography, and had a corresponding retention time on HPLC. Similar results were obtained in the HVPE in the above conditions (R_F 0.75 using arginine as a standard). Finally, intracisternally administered synthetic Tyr-Arg and endogenous peptide L3b′ had equipotent analgesic effects. These findings confirm that the endogenous analgesic compound, peptide L-3b′, is a dipeptide of amino acid sequence Tyr-Arg. In preliminary experiments, Tyr-Arg could also be isolated from the 0.4 M perchloric acid extract of the mouse brain, by which the proteolytic enzymes are denatured. It is interesting that the Tyr-Arg sequence is present in some hypothalamic hormones—residues 74–75 and 79–80 in human thyroid stimulating hormone, residues 78–79 in human luteinising hormone, and residues 5–6 in human, but not bovine,

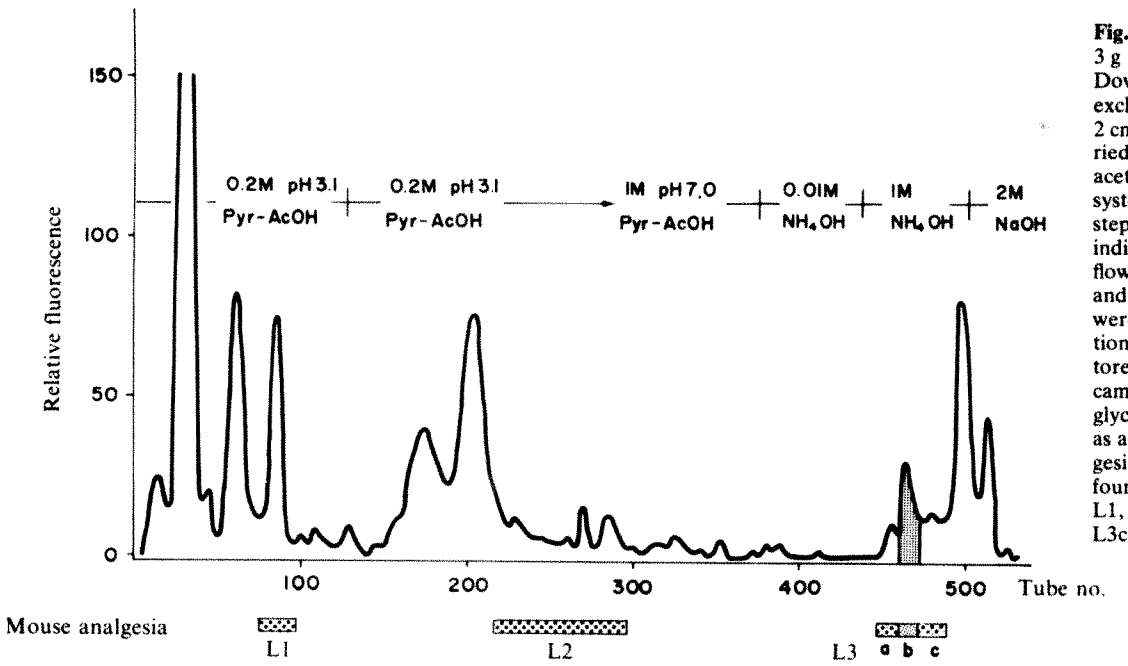


Fig. 2 Fractionation of 3 g of fraction L on a Dowex 50W-X2 cation exchange column (80 × 2 cm). Elution was carried out with a pyridine–acetate linear gradient system and using a stepwise system, as indicated. The rate of flow was 0.3 ml min^{-1}, and 15-ml fractions were collected. The elution pattern was monitored by the fluorescamine method with glycine (10^{-8} M = 100) as a standard. The analgesic activities were found in five fractions; L1, L2, L3a, L3b and L3c, as indicated by the horizontal bars.

β-melanotropin. It remains to be determined whether these hypothalamic peptides are precursors of the newly identified dipeptide.

When injected intracisternally, the analgesic effect of synthetic Tyr-Arg was dose dependent and was completely abolished by pretreatment with naloxone (0.1 mg per kg, s.c.). The ED_{50} value tested by the tail-pinch method was 34.7 nmol per mouse (11.7 μg per mouse) with 95% confidence limits of 22.0–54.9 nmol. Although the analgesic potency of Tyr-Arg was about 60 times less than that of morphine (ED_{50} 0.61 nmol per mouse), it was about 4.2 times higher than that of Met-enkephalin (ED_{50} 146 nmol per mouse). In addition, the analgesic effect of Tyr-Arg lasted longer (for 30 min at ED_{50} dose) than that of Met-enkephalin (disappeared within 15 min at ED_{50} dose). Tyr-Arg-induced analgesia was also demonstrated by the hot-plate method of Loh *et al.*[5], giving an ED_{50} of 15.7 nmol per mouse. Furthermore, Tyr-Arg showed analgesic activity in the rat when given into the nucleus reticularis gigantocellularis, an area of the brain which is highly sensitive to morphine analgesia[6].

Nevertheless, Tyr-Arg was only weakly inhibitory towards electrically induced contraction of longitudinal muscle of the guinea pig ileum (18.8% inhibition at 360 μM), suggesting that this compound does not interact with opiate receptors. We therefore studied the binding affinity of Tyr-Arg to specific opiate receptors. The binding assay, using the rat brain membrane preparation, was as described by Simantov *et al.*[7]. Tyr-Arg was only weakly inhibitory towards ^{3}H-dihydromorphine (24.9% inhibition at 100 μM) and ^{3}H-naloxone (22.7% inhibition at 100 μM), indicating that the dipeptide does not bind with the specific opiate receptors. This strongly suggests that the naloxone-reversible analgesic effect of Tyr-Arg is mediated by enkephalins or β-endorphin. Therefore, Tyr-Arg acts either as an enkephalin- or β-endorphin-releasing factor or as an inhibitor of the degradation enzymes of enkephalins, such as aminopeptidase and enkephalinase[8].

Met-enkephalin release was studied using the method described by Henderson *et al.*[9]. Its release from guinea pig striatal slices (four striata from two animals weighing ~600 mg) was detected by an opiate receptor binding assay after purification of the Met-enkephalin using HPLC as described by Meek and Bohan[10]. The basal release of Met-enkephalin in the superfusate (15-min collection period) was 16–17 ng. During 15 min of high-potassium ion (50 mM) stimulation, Met-enkephalin release increased to levels about four times higher than in the preceding spontaneous release samples. After 15 min exposure to a Tyr-Arg-containing medium (10^{-5} or 10^{-6} M) the output of Met-enkephalin increased to 2.3 times that of the preceding basal release (see Fig. 4).

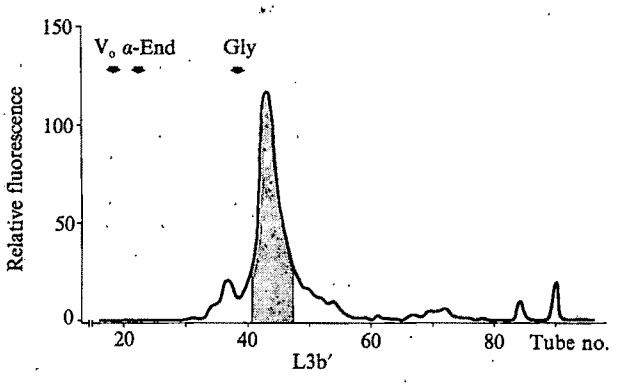


Fig. 3 Gel filtration chromatography of 8 mg of fraction L3b on a BioGel P-2 column (100 × 1 cm). Elution was carried out with 1 M acetic acid, the rate of flow was 0.2 ml min^{-1}, and 1.5-ml fractions were collected. The elution pattern was monitored by the fluorescamine method with glycine (10^{-8} M = 100) as a standard. The approximate elution positions of α-endorphin (α-End) and glycine (Gly) are indicated by the arrows. V_0 shows void volume.

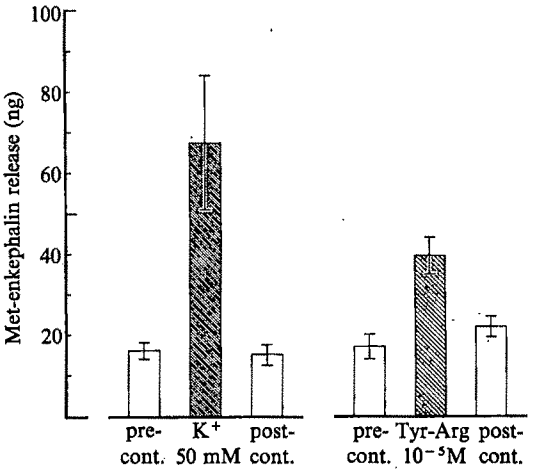


Fig. 4 Met-enkephalin release from rat striatal slices. Guinea pig striatal slices (500 μm thick, sagittal section) from four striata taken from two animals weighing ~600 mg were used in each experiment. The slices were superfused at a rate of 1 ml min^{-1} with a Krebs–bicarbonate medium at 37 °C, containing the dipeptides, Leu–Leu, Leu–Gly, Tyr-Tyr and Gly-Phe (each 1 mM) to protect against loss of released enkephalin by metabolism, and gassed with 95% O_2 and 5% CO_2. After an initial wash period of 15 min, superfusate samples were collected at 15-min intervals. For the first collection period, slices were superfused with normal medium containing the dipeptides (precontrol release). On the second collection period, slices were exposed to a high K^+ (50 mM) or Tyr-Arg (10^{-5} M)-containing medium (stimulated output) and the medium was replaced with normal solution for a 15-min period (post-control release). For the determination of the released Met-enkephalin, the method described by Meek and Bohan[10] was used. Final recovery of Met-enkephalin with this method was approximately 94%. The results are means ± s.e.m. for three or four experiments, as the absolute value of output from each striatal preparation.

In preliminary experiments, Tyr-Arg inhibited the enkephalin degradation enzymes in mouse brain homogenate preparations. However, its effect was weaker than that of bacitracin (less than 1/5th–1/10th, on a molar basis), which is a potent enkephalin degradation blocker. In addition, bacitracin did not produce analgesia even with intracisternal administration in a dose of 100 μg per mouse (71 nmol per mouse).

Thus, the analgesic effect of Tyr-Arg is interpreted as follows. Tyr-Arg itself does not bind with the opiate receptors but acts to release Met-enkephalin, which does bind with opiate receptors effectively, and consequently produces a potent analgesia. The relatively long-lasting analgesia induced by Tyr-Arg may be attributed to a stabilising effect on the released Met-enkephalin because Tyr-Arg weakly inhibits enkephalin degradation enzymes. Thus, Tyr-Arg seems to be a Met-enkephalin releasing factor, and its presence suggests the existence of a regulating mechanism for the release of Met-enkephalin. A preliminary report of a part of this work has been made elsewhere[11].

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Stimulation of the pituitary-adrenocortical axis by nerve growth factor

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Nerve growth factor (NGF) is a protein essential for the development and maintenance of the peripheral sympathetic nervous system[1,2], causing responsive neurones to increase in size and to extend neurites. Biochemically, the selective induction of tyrosine hydroxylase (TH) and dopamine β-hydroxylase[3,4] key enzymes in catecholamine biosynthesis, is one of its most characteristic effects. Both the morphological[5] and biochemical[6] effects are modulated by glucocorticoids, suggesting a close relationship between specific effects of NGF and hormone action. NGF has been shown to induce an increase in adrenal cyclic AMP in intact but not in hypophysectomised rats[7], and so we have looked directly at the effect of systemic administration of NGF on the hypothalamo-pituitary-adrenal axis. We report here that NGF induced an enhanced secretion of adrenocorticotropin (ACTH) and a prolonged increase in plasma glucocorticoid concentration after intravenous (i.v.) injection. Such effects could have important implications for the biological activity of NGF.

NGF was prepared from submaxillary glands of adult male mice by the method of Bocchini and Angeletti[8], as the 2.5S subunit, with the modifications of Suda *et al.*[9]. The purity of NGF was controlled by SDS gel electrophoresis and its biological activity was determined as described by Fenton[10]. The biological activity of our NGF preparation was 250 biological units per μg of protein. Biologically inactive NGF was prepared by oxidation of the tryptophan residues with *N*-bromosuccinimide as described by Frazier *et al.*[11]. Adult male Sprague–Dawley rats of 100–120 g body weight were maintained at 24 °C, five per cage, with lights on at 0700 h and off at 1900 h. Food and water were provided *ad libitum*. For hormone studies they were injected in the tail vein with NGF at between 0700 h and 0900 h. They were killed by decapitation and trunk blood was collected at intervals of 5–720 min. Plasma ACTH and corticosterone were determined by radioimmunoassay as previously described[12]. The ACTH antibody used cross-reacts completely on a molar basis with $ACTH_{1-24}$ but not (cross-reaction <0.005%) with α-melanocyte-stimulating hormone (α-MSH), β-MSH, $ACTH_{17-39}$ or β-endorphin. There was no cross-reactivity with NGF. TH activity was determined according to Mueller *et al.*[13].

Table 1 Specificity of NGF for hormonal changes in rat plasma

	ACTH (pg per ml)	Corticosterone (μg per 100 ml)
Controls	576 ± 110	3.5 ± 1.1
Saline	609 ± 142	4.1 ± 1.2
NGF	1,223 ± 113*	17.5 ± 1.8*
Cytochrome *c*	582 ± 138	4.7 ± 1.6
Inactive NGF	343 ± 145	3.1 ± 1.1

Groups of five rats were injected i.v. with saline or equimolar concentrations (10 pmol per g BW) of various proteins. Rats were killed 90 min later and plasma ACTH and corticosterone were measured[12]. Results are mean ± s.e.m.

* $P < 0.005$, compared with controls (saline or cytochrome *c*) or with inactive NGF.

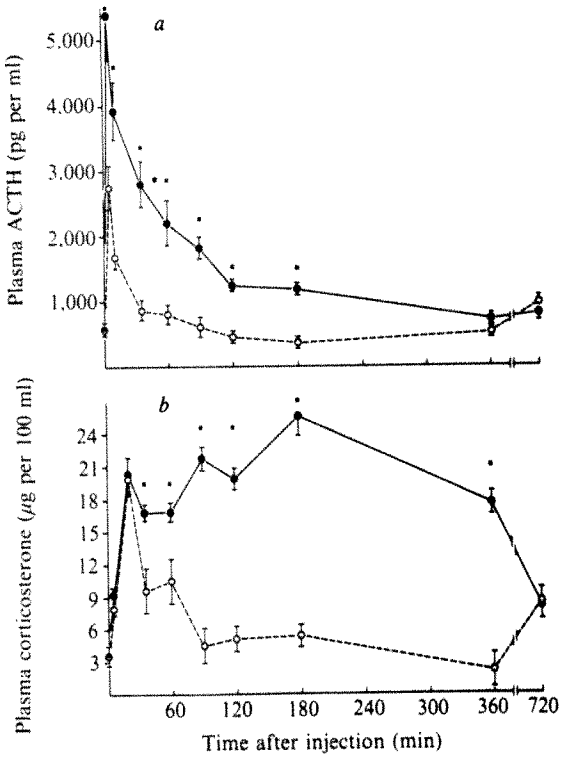


Fig. 1 Stimulation of the pituitary-adrenocortical axis by NGF. Groups of 5–10 rats were injected i.v. with 100 pmol per g NGF (●) or equimolar amounts of cytochrome *c* (○). They were killed at the times indicated and plasma was assayed for ACTH (*a*) and corticosterone (*b*) by radioimmunoassay[12]. Values represent the mean ± s.e.m.

* $P < 0.0025$, compared with cytochrome *c* controls.

A single i.v. injection of NGF (100 pmol per g) produced a prolonged stimulation of the adrenocortical system (Fig. 1). NGF increased plasma ACTH levels from 576 ± 110 pg ml^{-1} in controls to 5,380 ± 690 pg ml^{-1} ($n = 10$) at 5 min. ACTH did not return to resting levels within 180 min. Plasma corticosterone levels continued to increase for 20 min and remained at a high plateau of 20–24 μg per 100 ml for at least 6 h. The specificity of the response to NGF was confirmed by injecting control animals with equimolar concentrations of cytochrome *c*, a protein similar to NGF in molecular weight and isoelectric point. The

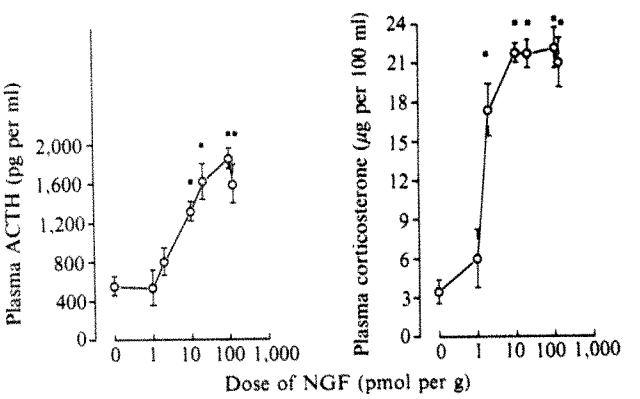


Fig. 2 Effect of concentration of NGF on stimulation of pituitary-adrenocortical axis. Groups of five rats were injected i.v. with NGF at 1.0–300 pmol per g. They were killed 90 min later and plasma was assayed for ACTH and corticosterone[12]. Values are expressed as mean ± s.e.m.

* $P < 0.005$, compared with cytochrome *c* controls.

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animals showed only hormonal changes of short duration (Fig. 1): plasma ACTH increased ($2,750 \pm 349$ pg ml^{-1}, $n = 10$) at 5 min, returning to resting levels within 35 min, and plasma corticosterone levels peaked at 20 min, returning to normal within 90 min. Thus the systemic application of NGF causes prolonged stimulation of the pituitary-adrenocortical axis that clearly differs from the acute short-lasting response to cytochrome *c*.

Injection of biologically inactive NGF had no significant effect on plasma ACTH or corticosterone levels (Table 1), indicating that only the biologically active molecule can activate the pituitary-adrenocortical axis. The hormonal response to biologically active NGF is dose-dependent (Fig. 2). The minimum dose that resulted in a significant ($P < 0.005$) increase in plasma ACTH and corticosterone 90 min after injection was 10 pmol per g. Maximum effect was achieved with concentrations 10 times greater.

Table 2 Effect of NGF on TH activity in sympathetic ganglia of rats

Treatment	TH activity (nmol dopa per h per mg protein) Superior cervical ganglion	Coeliac ganglion
Saline	1.2 ±0.06	0.95±0.12
NGF	1.71±0.15*	2.51±0.17†
Inactive NGF	1.0 ±0.05	1.01±0.05

Groups of six rats were injected i.v. with saline or equimolar concentrations (10 pmol per g) of biologically active or inactive NGF. TH activity was determined 48 h later[13] by the amount of 3,4-dihydroxyphenylalanine (dopa) formed.

* $P < 0.025$, † $P < 0.01$ } Compared to animals treated with inactive NGF or saline.

The minimum dose of NGF (10 pmol per g) required to induce hormonal changes in plasma is identical to that necessary to elicit a significant ($P < 0.025$) induction of TH in sympathetic ganglia (Table 2). Thus NGF-mediated hormonal changes could have important implications for the initiation of selective induction of TH activity in sympathetic neurones. We do not know whether these hormonal changes are of peripheral or central origin. However, the short latency of the increase in plasma ACTH may indicate direct action on the hypothalamo-pituitary system, as suggested by the observations of Nagaiah *et al.*[14]. Our findings, together with the observation that intracerebral injection of NGF induces an increased drinking response[15,16], indicate that NGF can affect both central and peripheral nervous systems. The significance of the hormonal changes with respect to the physiological role of NGF remains to be elucidated.

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Evidence for thyroxine–growth hormone interaction during brain development

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Growth-promoting hormones have complementary and synergistic effects on cell growth. In particular, thyroid hormone–growth hormone (GH) interactions are important in the regulation of somatic development[1]. Although the requirement of thyroid hormone for normal brain development is well established[2], the effects of growth-promoting peptide hormones, including GH, nerve growth factor and insulin, on central nervous system development have not been well characterised. To investigate the possibility of a role for GH in normal CNS development, we have measured its effect on the activity of brain ornithine decarboxylase (ODC), a key enzyme in the synthesis of the polyamines, spermidine and spermine[3,4]. Previously, we reported increased ODC activity in neonatal rat brain after intraperitoneal (i.p.) or intracranial administration of GH and other growth-promoting peptide hormones[5], results which have been supported by other data[6,7]. We now extend our findings to show that GH stimulation of brain ODC activity depends on the presence of thyroid hormone, even though thyroid hormone, by itself, does not stimulate ODC activity in brain. This is in contrast to liver, where GH and thyroid hormones seem to stimulate ODC activity independently. Our results suggest that thyroid hormone selectively alters or modulates the action of GH in mammalian brain and that abnormal brain development observed in hypothyroidism may in part result from decreased expression of GH activity.

One-day-old Sprague–Dawley rats (Zivic–Miller, Allison Park, Pennsylvania) were thyroidectomised (thyrx) by treatment with 100 μCi of $Na^{131}I$ (ref. 8). At 18 d, normal and thyrx rats were injected with saline (control), thyroxine (T_4), bovine GH (bGH), or T_4 and bGH (for details see Table 1 legend). ODC activity was determined *in vitro* as $^{14}CO_2$ produced from [1-^{14}C]ornithine[3,6]. At 18 d, body and brain weights of the thyroid-deficient rats were significantly reduced compared with controls; the body weights of thyrx and control rats averaged 37.5 ± 1.7 g (mean ± s.e.m.) and 42.7 ± 0.5 g ($P < 0.05$), respectively, and respective brain weights averaged 1.43 ± 0.03 g and 1.58 ± 0.03 g ($P < 0.02$).

In normal rats, i.p. bGH stimulated brain ODC activity twofold (217% of control, Table 1, expt 1), a finding similar to that observed in previous studies[5]. Although T_4 alone did not significantly increase brain ODC activity (expt 2), stimulation by T_4 and bGH given together was equivalent to that by bGH alone (expt 1).

The basal ODC activity of thyrx control (saline-treated) rats was elevated (158%) compared with normal controls (expt 2). This may have resulted from post-thyroidectomy delay in the normal decline of ODC activity from high perinatal to low adult levels[10], similar to the hypothyroidism-induced delay of brain maturation observed by others[2]. As in normal animals, brain ODC activity of thyrx rats was not significantly altered by T_4 treatment (129 ± 17% of thyrx controls). Importantly, bGH also failed to increase ODC activity in thyrx rats, and administration

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Table 1 Effects of thyroxine and thyroidectomy on bGH stimulation of brain ODC activity

	Treatment	ODC activity	% of control	P
Expt 1	Control	722 ± 28 (3)	—	—
Normal rats	bGH	1,568 ± 211 (4)	217 ± 29	<0.02
	T_4 + bGH	1,464 ± 159 (5)	203 ± 22	<0.001
Expt 2	Control	817 ± 83 (8)	—	—
Normal rats	T_4	1,036 ± 92 (5)	127 ± 11	NS
Thyrx rats	Control	1,294 ± 122 (7)	158 ± 15*	<0.01*
	T_4	1,663 ± 218 (8)	129 ± 17	NS
	bGH	1,391 ± 146 (9)	108 ± 11	NS
	T_4 ± bGH	2,844 ± 196 (9)	220 ± 15	<0.001

Normal and thyroidectomised (thyrx) rats received saline (control), T_4 (20 μg per 100 g body weight) i.p. at 28, 19 and 4 h before death, bGH (300 μg per 100 g body wt) i.p. 4 h before death, or T_4 plus bGH. bGH (NIH-GH-B16) was chromatographed before use to obtain a monomer fraction[20]. ODC activity (d.p.m. per 30 min per g wet weight) is given as mean ± s.e.m., with the number of animals in parentheses. NS, Not significant.

* Compared with normal control.

of T_4 before bGH completely restored the ODC response, with an activity increased to 220% of control levels. This was comparable to the normal response to bGH (217% of control) seen in euthyroid rats.

This effect of T_4 was not apparent in liver (Table 2). As noted by us and others[5,11], GH treatment of normal rats produced a large (148-fold) increase in liver ODC activity (expt 1). T_4 increased liver ODC activity eightfold in normal and thyrx rats (expt 2), and the increase seen after T_4 and bGH in normal rats did not differ from that of bGH alone (expt 1). In contrast to results with brain, liver ODC activity in thyroid-deficient rats was stimulated by bGH (37-fold increase, expt 2). Furthermore, the response of T_4 plus bGH (48-fold increase) was similar to that for bGH alone. As shown in Table 2, after bGH, or T_4 plus bGH, the stimulation of ODC activity was greater in normal rats than in thyrx rats. This may reflect a decrease in liver responsiveness to GH after thyroidectomy which was not adequately corrected by T_4 administration. It is clear, however, that in contrast to its effect on brain, GH stimulated liver ODC activity in thyrx rats without T_4 pretreatment.

We conclude that an action of T_4 is required for the stimulation of brain ODC by bGH. The mechanism for this 'permissive' (ref. 12) or modulating action of T_4 is unknown. It is possible that the number of receptors for GH or for a GH-dependent agent such as somatomedin[13] is reduced in thyroid deficiency in a manner similar to that reported for prolactin[14] and catecholamine receptors[15]. Alternatively, thyroxine may regulate the availability of GH-dependent (perhaps brain-specific) somatomedin or somatomedin-like factors. The difference between brain and liver response might also result from differential effects of T_4 on ODC isoenzymes, the existence of which is suggested by kinetic data[16].

Because, in severe hypothyroidism, the amount of GH in plasma is greatly reduced[17], attempts have been made to correct the GH deficiency in thyroid-deficient neonatal rats with large amounts of exogenous hormone[18,19]. Interestingly, this treatment partially reversed the abnormal pattern of neonatal brain growth. Thus, in neonatal hypothyroidism, abnormal brain development might result in part not only from decreased GH or GH-dependent growth factors, but also, as suggested by the present study, from a lack of thyroid hormone support for the limited GH present.

As ODC activity and polyamine synthesis seem to be intimately associated with the regulation of nucleic acid and protein synthesis[3,9], the increase in brain ODC activity following GH administration suggests a role for GH in the regulation of brain development. It should be recognised, however, that in this and previous work[5], ODC activity has been used as an indicator of growth stimulation[3]. It remains to be determined whether the regulatory actions of GH on brain development are mediated by changes in polyamine synthesis.

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Table 2 Effects of thyroxine and thyroidectomy on bGH stimulation of liver ODC activity

	Treatment	ODC activity	Treated / Control	P
Expt 1	Control	483 ± 186 (3)	—	—
Normal rats	bGH	71,343 ± 8,184 (4)	148-fold ± 17	<0.001
	T_4 ± bGH	57,691 ± 8,879 (5)	119-fold ± 18	<0.001
Expt 2	Control	403 ± 117 (5)	—	—
Normal rats	T_4	3,005 ± 581 (4)	8-fold ± 1	<0.01
Thyrx rats	Control	692 ± 271 (5)	1.7-fold ± 1*	NS*
	T_4	5,715 ± 2,168 (5)	8.3-fold ± 2	<0.05
	bGH	25,707 ± 6,181 (5)	37-fold ± 9	<0.01
	T_4 + bGH	33,288 ± 7,642 (5)	48-fold ± 11	<0.001

Details same as for Table 1.

Apamin blocks certain neurotransmitter-induced increases in potassium permeability

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Apamin[1] is a neurotoxic polypeptide of known structure[2,3] isolated from bee venom. Shuba and coworkers[4,5] have recently shown that it abolishes the hyperpolarising action of externally-applied ATP on visceral smooth muscle (guinea pig stomach and taenia coli) as well as the hyperpolarisation (inhibitory junction potential) that follows stimulation of the non-adrenergic inhibitory nerve supply to these tissues. As it has been proposed[6] that

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ATP is the neurotransmitter involved in the latter response, Vladimirova and Shuba[4] tentatively concluded that apamin is a specific postsynaptic blocking agent of this non-adrenergic, possibly 'purinergic', inhibition. We have confirmed the important observation that nanomolar concentrations of apamin reduce inhibition by ATP and by non-adrenergic nerve stimulation, but further experiments suggest that, rather than acting as a specific blocker of ATP receptors, apamin inhibits the increase in potassium permeability caused by a number of agents, including ATP.

The first new finding (Fig. 1*a*) was that apamin also reduces the inhibition produced by stimulation of the sympathetic (perivascular) nerve supply to the taenia coli. As the transmitter in this case is noradrenaline[7], it seemed possible that apamin interferes with some of the actions of catecholamines as well as of ATP. In keeping with this, inhibition of the taenia by the selective α-adrenoreceptor agonist amidephrine is also blocked by apamin. Figure 1*b* shows the noncompetitive nature of this antagonism, the maximum inhibition by amidephrine being reduced by apamin at concentrations as low as 1–3 nM. In each of four other such experiments, 5 nM apamin was sufficient to abolish amidephrine inhibition. These results contrast with the competitive antagonism seen with α-adrenoreceptor blockers in this tissue[8], and suggest that apamin acts at a stage beyond the recognition site of the receptor. As both α-adrenoreceptor-mediated inhibition[9–11] and the inhibitory response to ATP[12,13], as well as to non-adrenergic nerve stimulation[14] are due mainly to an increase in the potassium permeability of the smooth muscle membrane, it seemed possible that apamin could act by preventing the rise in potassium permeability rather than by blocking the receptors that initiate it.

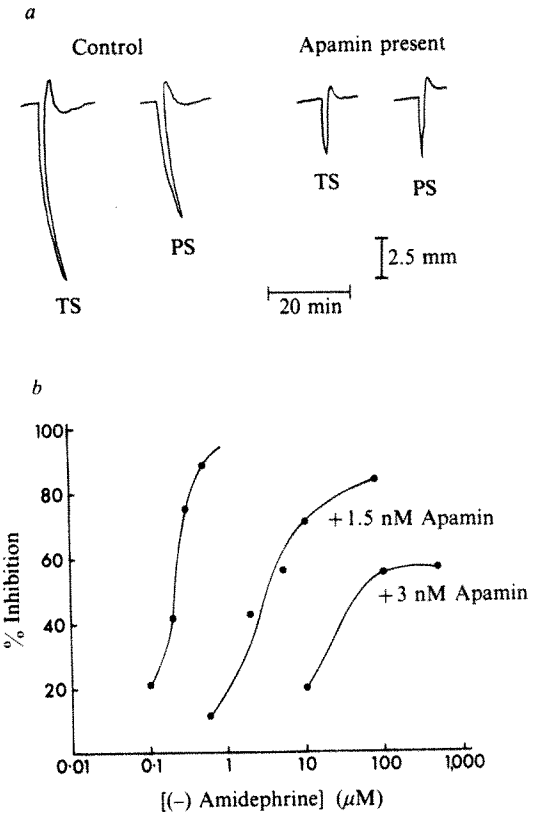


Fig. 1 *a*, Apamin (5 nM) reduces the relaxation caused by both transmural (TS) and perivascular (PS) stimulation of the nerve supply to an isolated strip of guinea pig taenia coli. Atropine (3 μM) present throughout. Stimulation parameters: supramaximal square pulses (200 μs) for 30 s at either 5 (TS) or 20 Hz (PS). Isotonic recording. *b*, Apamin antagonises the inhibition by the α-adrenoreceptor agonist amidephrine of the contractile response of a taenia strip to carbachol (20 nM). For details of the experimental procedures for *a* and *b*, see refs 7 and 8, respectively.

This hypothesis was tested using another tissue, guinea pig liver, in which α-adrenoreceptors also enhance potassium permeability, as indicated by increases in potassium flux, membrane potential and conductance, and in addition a net loss of potassium[15,16]. ATP also hyperpolarises guinea pig liver cells, probably by the same mechanism[17]. The response can be conveniently studied using a suspension of isolated hepatocytes, as the loss of potassium following a substantial increase in potassium permeability is readily detected by means of a potassium-sensitive electrode in the suspension[16,18]. This is shown in Fig. 2, which also shows that apamin greatly reduces the potassium loss, and hence, by inference, the increase in potassium permeability caused by both ATP and noradrenaline. In this respect apamin resembles quinine[18], which is, however, approximately 10^5 times less active.

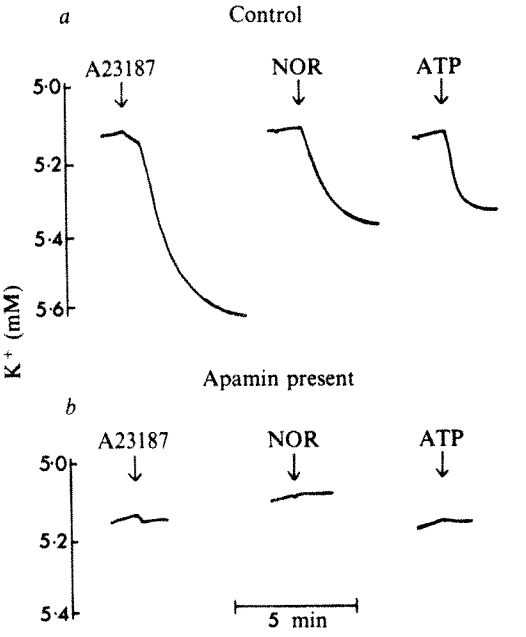


Fig. 2 *a*, A23187 (5 μM), (−)noradrenaline (5 μM, in the presence of propranolol at 5 μM) and ATP (1 μM) cause K loss from isolated guinea pig hepatocytes. The movement of K between the cells and the incubation medium was followed by means of a K-sensitive electrode in the cell suspension (which contained ~50 mg cells in 2 ml and was stirred). A downward deflection indicates an increase in external K concentration, corresponding to loss of K from the cells. For other details, see ref. 18. *b*, Apamin (10 nM, applied 2–3 min beforehand) greatly reduces the response to all three agents applied to other samples from the same batch of hepatocytes.

The rise in potassium permeability is thought[18–20] to be triggered by an increase in the concentration of ionised calcium in the cytosol of the hepatocytes, and this suggested a means of testing whether apamin blocks the receptors or the consequences of receptor activation. If the former is the case, the change in calcium movement should also be abolished. This was studied using noradrenaline as the agonist, and the results are shown in Fig. 3. Apamin at concentrations sufficient to eliminate the potassium movement induced by noradrenaline caused little change in the rapid calcium loss that follows the application of α-agonists to hepatocytes[21,22]. Thus the receptors are still functional. The same dissociation between potassium and calcium movements was seen with the divalent ionophore A23187, which in the presence of apamin no longer caused potassium loss (Fig. 2) although still initiating calcium movement[18]. A further test of the same point was made with rabbit liver slices which respond to α-agonists with increases in ^{45}Ca and ^{42}K efflux and a rise in glucose release[19]. Apamin (10 nM)

abolished the effect on ^{42}K efflux, whereas the changes in ^{45}Ca efflux and glucose release were unaltered.

The conclusion that apamin is not a receptor blocker is strengthened by our finding that it does not inhibit the contractile response of the isolated rat vas deferens preparation to ATP or to α-agonists. Nor are the actions of ATP on histamine release from rat peritoneal mast cells impaired (B. Gomperts, personal communication).

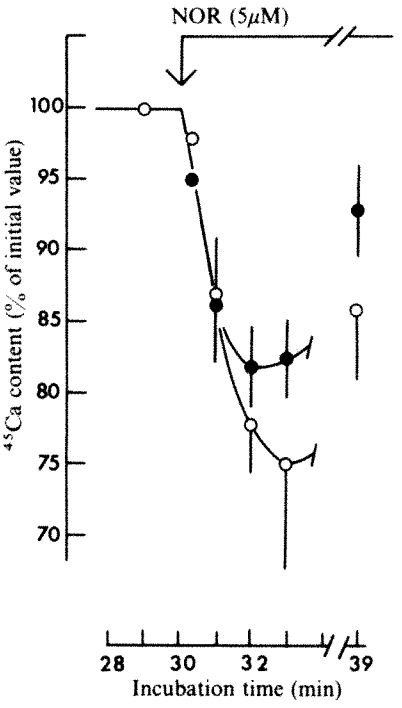


Fig. 3 Noradrenaline (5 μM, in the presence of propranolol at 5 μM) causes isolated guinea pig hepatocytes to lose calcium, and the initial loss is little affected by apamin. Open circles, controls; closed circles, cells treated with apamin at either 10 (4 expts) or 100 nM (1 expt), applied 3 min before noradrenaline. Calcium contents were determined by equilibrating the hepatocytes with ^{45}Ca (total external calcium, 1.8 mmol l^{-1}) for 30 min and then counting the ^{45}Ca content of the pellet after centrifuging the cells through an oil phase, as described in ref. 18. The values (ordinate) have been expressed as percentages of the content before noradrenaline (mean value, 1.91 ± 0.08 (s.e.m., $n = 5$) nmol calcium per mg dry weight). The vertical bars indicate the s.e.m.s of 4–5 observations. Abscissa, incubation time in ^{45}Ca.

It is also clear that apamin is highly selective in that only some potassium permeabilities are blocked. Thus Vladimirova and coworkers have shown[5] that concentrations of apamin much greater than those used for their smooth muscle experiments have little effect on the frog neuromuscular junction, or on synaptic inhibition in an identified molluscan neurone. Other actions that we have found to be unaffected by apamin at up to micromolar concentrations include: (1) the inhibitory effect of acetylcholine on the beat of the isolated perfused rabbit heart; (2) the increase in potassium permeability that follows a rise in intracellular calcium in human red blood cells; and (3) inhibition of the mechanical activity of the guinea pig taenia coli by β-adrenoreceptor agonists and by low concentrations of adenosine and AMP. This last finding is in keeping with other evidence (see refs 23, 24) that inhibition of the taenia coli by β-adrenoreceptors, as well as by the sub-type of purinoceptor which is activated by adenosine and by AMP, does not involve the increase in potassium permeability seen with α-adrenoreceptor agonists and ATP.

In conclusion, apamin abolishes certain responses to ATP and to α-adrenoreceptor agonists by inhibiting the increase in potassium permeability that these agents initiate. The simplest explanation is that apamin blocks the potassium channels involved, although it is also possible that it interferes with the mechanism by which a rise in cytosolic calcium causes these channels to open. The potency and selectivity of apamin should make it useful for the study of neurotransmitter-controlled potassium conductances. In addition, the finding that apamin blocks calcium-mediated increases in the potassium permeability of hepatocytes but not red blood cells suggests that there may be several types of calcium-dependent potassium channel.

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Induction and kinetics of natural killer cells in humans following interferon therapy

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Natural killer (NK) cells are non-B, non-T lymphocytes that effect spontaneous cytolysis of both virus-infected and neoplastically transformed target cells[1,2]. These NK lymphocytes have been detected in several species including man[3–9]. Interferon is a primary regulator of natural killer activity[6,10,11]. Because NK cells have been implicated in the regulation of tumour cell expression and can be induced by interferon in murine models, we have studied patients receiving large doses of interferon to determine (1) whether interferon could induce NK lymphocytes in the peripheral blood of man, and (2) whether there are characteristic kinetics for the appearance, disappearance and reactivation of NK lymphocytes following interferon therapy.

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We report here the activation of human NK cells by the systemic inoculation of human subjects with interferon. Five patients received interferon as therapy for non-Hodgkin's lymphoma. All showed a marked increase in NK cell activity 12–24 h after inoculation. Peak NK activity occurred 18 h after introducing interferon, and thereafter declined rapidly but remained above pre-interferon levels. Induced NK activity occurred with reintroduction of interferon but at lower levels of activity and with different kinetics.

The interferon used in this study was from a human leukocyte interferon preparation containing 1×10^6 units of interferon per mg protein. Patients receiving interferon had non-Hodgkin's lymphoma. (For more details about the interferon preparation, its administration, the diagnostic criteria and clinical course of the non-Hodgkin's lymphoma patients, see ref. 12.) Four (W.M., P.K., B.S., J.B.) of the patients were treated with 10×10^6 units of interferon intramuscularly for 7–30 days, and one patient (GL.) received 5×10^6 units for 2 days, followed by 10×10^6 units daily for the next 27 days. Of these patients only P.K. had received interferon previously. Data for NK activity are expressed in terms of the cytotoxicity index (CI). This allows each patient to act as his own control throughout all observations. The CI was determined by dividing the specific killing of MOLT-4 target cells at a given experimental time by the maximum killing of MOLT-4 target cells observed for that patient's lymphocytes during the entire course of observations. The CI values for each of the patients before interferon therapy were 0.16, 0.08, 0.02, 0.14 and 0.5, respectively (Fig. 1). Lysis was detected in a 6-h ^{51}Cr release assay of triplicate samples, with variance among triplicates of less than 10%. Lymphocytes from the two patients (W.M. and P.K.) with the highest CI values had been tested against various targets including MOLT-4 cells, HeLa cells uninfected or persistently infected with measles virus, HT-29 cells and Daudi cells. The characterisation, handling, passage and ^{51}Cr-labelling of these cells have been reported elsewhere[13,14]. Killing by lymphocytes from several normal controls and from these two patients was effective against the various targets, but more MOLT-4 cells were lysed than other target strains, and so thereafter, only ^{51}Cr-labelled MOLT-4 cells were used. These cells ($1-2\times10^4$ cells ml^{-1}) were mixed with varying amounts of lymphocytes collected from each patient's peripheral blood by Ficoll–Hypaque passage, depleted of adherent cells[14], and used at ratios of 1:10, 1:20 and 1:40. To ensure internal consistency throughout the various time points assayed, mean values of spontaneous ^{51}Cr release (range 10–23%) were calculated and results corrected at each point as described[14].

As shown in Fig. 1, enhanced NK lymphocyte activity was not present at 6 h, but appeared at 12 h and peaked at 18 h after the initial injection of interferon. Thereafter, killing activity fell rapidly but stayed above pre-interferon levels. Lymphocyte killing generated by interferon therapy was caused by lymphocytes which expressed Fc receptors. Patients (W.M. and P.K.) had less than 1% of the total killing activity in Fc-negative lymphocytes. Mean specific ^{51}Cr release ±1 s.d. due to total peripheral blood lymphocytes compared with adherent cell-depleted Fc-positive and Fc-negative populations was: patient W.M., total lymphocytes 44±6%, Fc positive, 32±1%, Fc negative 3±0.3%; patient P.K., total lymphocytes 28±6%, Fc positive 17±0.2%, Fc negative, 0%. Thus, because (1) lymphocytes bearing Fc-positive receptors performed the killing, (2) many different target cell lines were lysed, and (3) cytotoxicity was induced by interferon, these killer cells can be characterised as NK lymphocytes[1,2,15,16].

We were interested in whether NK lymphocyte killing responses could be restimulated in patients who had previously been given interferon therapy. Two patients (J.B., B.S.) received 10×10^6 units interferon daily for 7 days, and were taken off therapy for 46 and 73 days, respectively. They were then given 10×10^6 units interferon daily for 9 or 30 days, respectively. In patient B.S., whose CI was 0.1, the initial course of interferon increased NK activity to a CI of 1.0 by 18 h. However, the

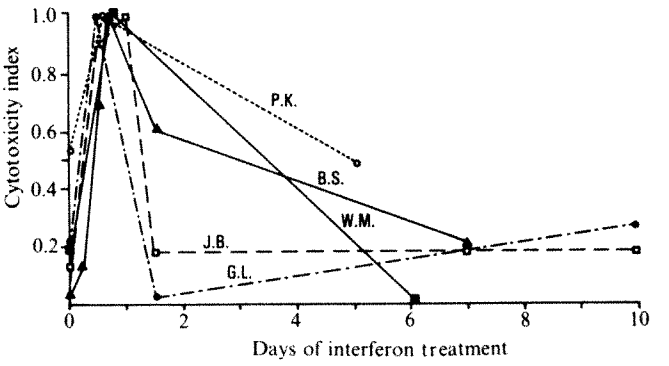


Fig. 1 The cytotoxic indices of peripheral blood lymphocytes (PBLs) from five patients treated with 10×10^6 units interferon daily plotted as a function of time. Peripheral blood lymphocytes, sampled before interfero therapy and at the indicated intervals thereafter, were assayed for spontaneous cytolytic activity against MOLT-4 cells in a standardised 6-h ^{51}Cr-release assay at lymphocyte: target ratios of 5:1, 20:1 and 40:1. The cytotoxic indices were calculated from % specific killing data at a PBL:target ratio of 40:1 by dividing the % specific killing at a given time by the maximum % specific killing observed for that patient during treatment. % Specific killing data represented the mean of at least three replicates and were corrected for variance in the spontaneous releases, which ranged from 6 to 24%. Cytotoxic indices for normal subjects assayed concurrently had a mean of 0.9 and the minimum CI seen in a single normal subject was 0.5.

second interferon course generated no NK activity. Patient J.B. did develop NK lymphocyte activity 73 days after reinoculation with interferon (CI 0.2), but at fivefold lower levels than those generated during the primary inoculation of interferon. When interferon therapy was attempted a third time in patient J.B. (10×10^6 units of interferon given daily for 30 days) 150 days after cessation of the second course, a significant rise in NK activity was seen (CI 0.5). Further, the elevated NK response observed in P.K. (Fig. 1) to interferon occurred 1.5 yr after a prior course of leukocyte interferon. Throughout these studies there was no extension of the patients' lymphoma and no introduction of chemotherapy. Therefore, neither haematological complications of conventional chemotherapy nor a worsening of their disease can explain the transient refractoriness of B.S. and J.B. to repeated interferon induction.

Recently, patients with non-Hodgkin's lymphoma underwent remission in both size and distribution of their lymphadenopathy after intensive therapy with interferon[12]. Prevention of relapse has also been reported in patients with osteosarcoma receiving interferon[17]. We have noted the successful induction of NK lymphocyte activity in patients with non-Hodgkin's lymphomas following interferon treatment. Hence, in humans, like mice, interferon induces NK activity. The role of induced NK activity against the patient's own tumour cells is now under investigation.

After completion of these studies, we became aware that Einhorn, Blomgren and Strander[18] had also demonstrated acute enhancement of NK responses in four of five tumour patients given a single injection of 3×10^6 units of human leukocyte interferon.

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Desensitisation of cultured glial cells to epidermal growth factor by receptor down-regulation

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Epidermal growth factor (EGF), which can be purified from the mouse submaxillary gland[1] or from pregnant human urine[2], is a potent multiplication-stimulating factor for several types of cultured cells, including human fibroblasts[2,3] and glial cells[4]. The molecule binds with high affinity and saturation kinetics to a cell-surface receptor[3], is subsequently internalised[5,7] and finally degraded[5,6]. The binding event is accompanied by a reduction in the number of EGF receptors[5,8]. This phenomenon—'receptor down-regulation'—has been demonstrated with several hormones and may be a general principle for the modulation of binding groups on the outer cell surface[9,10]. Further, it has been proposed that receptor loss acts to regulate the cellular response to the binding ligand[11]. The present study provides direct experimental support for this hypothesis. It demonstrates that down-regulation of EGF receptors on glial cells causes desensitisation of the mitogenic response of these cells to subsequent stimulation with EGF.

Attempts to demonstrate cellular desensitisation, that is, a decreased metabolic response to a factor, may be hampered by the fact that the initial exposure to the factor is likely to produce significant changes in the basal level of the metabolic parameter in question. The present experiments were designed to dissociate the event of down-regulation of EGF receptors on glial cells from the mitogenic response of these cells to EGF. Treatment of glial cells with increasing concentrations of EGF (up to 167 nM) in Ham's F-10 medium for 24 h resulted in progressive down-regulation of the EGF receptors; loss of about 90% of the EGF receptors could be induced, as revealed by Scatchard analysis[12] of binding experiments with ^{125}I-labelled EGF (Fig. 1). This finding was expected by analogy with previous data on EGF receptors of other types of cultured cells[5,8]. In contrast, EGF was incapable of inducing a mitogenic response in the presence of plain F-10 medium; only after supplementation with human serum albumin (HSA) was this medium permissive to multiplication-stimulating activity (Table 1). Pre-exposure of glial cells to plain F-10 medium did not affect the time (about 13 h) required for subsequent recruitment of cells to DNA synthesis on stimulation with EGF in the permissive medium (F-10 medium supplemented with HSA) (Fig. 2). This suggests that the block in the mitogenic response to EGF imposed by the non-permissive conditions was probably at an early step close to the ligand–receptor binding event and preceding the reactions leading to commitment of the cells to enter the cell cycle.

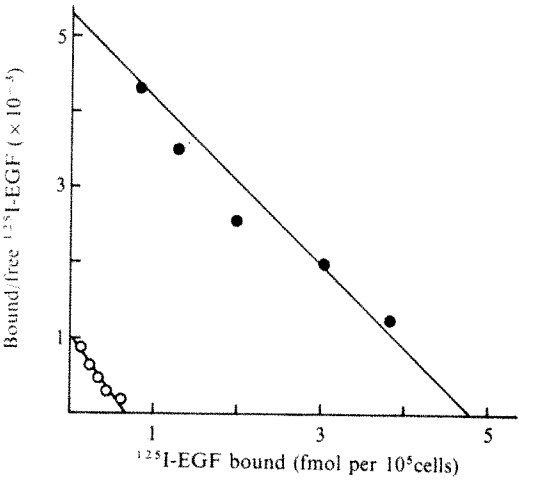


Fig. 1 Effect of EGF pretreatment on ^{125}I-EGF binding to human cultured glial cells. Human normal glia-like cells, line 787 CG (refs 16, 17), were seeded on 2.5-cm dishes in 2 ml of Eagle's medium containing 10% fetal calf serum. After 48 h at 37°C the medium was replaced with 2 ml of Ham's F-10 without serum. The cultures (approximately 1.1×10^5 cells per dish) were then incubated for 22 h in 2 ml of Ham's F-10 medium in the absence (●) or presence of 167 nM EGF (○). Cells were then washed once with 2 ml of plain F-10 medium and five times with F-10 medium containing 1 mg ml^{-1} of HSA. The washed cells were incubated for 2 h at 4°C with various doses of ^{125}I-EGF in 1 ml of F-10 medium containing 1 mg ml^{-1} HSA. The cultures were then washed six times with 2 ml of ice-cold phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, *p*H 7.3) containing 1% fetal calf serum. Cells were lysed in 1 ml of 0.3 M NaOH and the cell-bound radioactivity determined in a Packard Auto-Gamma scintillation spectrometer. Nonspecific binding, determined in the presence of excess unlabelled EGF (about 1 μM) was subtracted. Binding data were treated by Scatchard analysis[12].

Table 1 Multiplication-stimulating activity of EGF on glial cells in F-10 medium, with or without human serum albumin

Addition	Multiplication-stimulating activity (c.p.m. ^{3}H)
None	814
EGF (1.6 nM)	1,121
HSA (1 mg ml^{-1})	2,716
HSA (1 mg ml^{-1}) + EGF (1.6 nM)	10,926

Multiplication-stimulating activity was estimated using the incorporation of ^{3}H-thymidine into trichloroacetic acid-precipitable material of serum-deprived sparse cultures of a human normal glia-like cell line[16,17] as described elsewhere[18].

Cell cultures in which the EGF receptor number had been reduced by preincubation with EGF (in non-permissive medium) were less responsive to further exposure to EGF (in the permissive medium). The dose–response curve for EGF (based on the increase in labelling index) was more than fivefold shifted to the right in these cells compared with controls with normal EGF receptors (Fig. 3). The maximal response obtained at saturating concentrations of EGF was only slightly lower than in control cultures. Apparently, the post-receptor pathway in EGF-induced mitogenesis did not become limiting by preincubation with EGF; rather, desensitisation occurred by reduction in receptor number. Maximal biological response, demonstrable with only a fraction of the total number of receptors occupied, indicates the presence of spare receptors for EGF on glial cells, in accordance with previous data[13].

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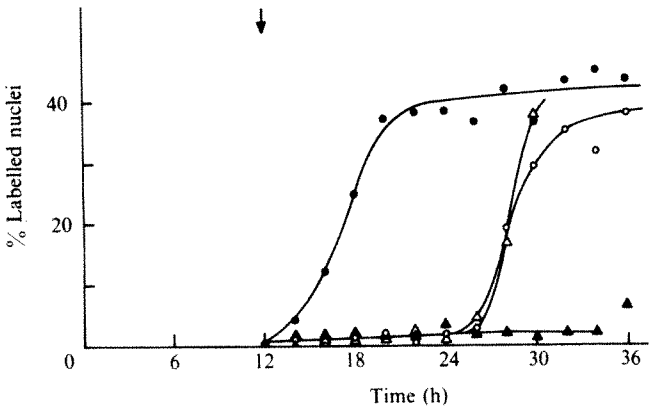


Fig. 2. Effect of EGF pretreatment on the time required for recruitment of glial cells to DNA synthesis on stimulation with EGF. Human normal glia-like cells, line 787 CG (refs 16, 17), were seeded on 2.5-cm dishes in 2 ml of Eagle's medium, containing 10% fetal calf serum ($\sim 10^5$ cells per dish). After 24 h at 37 °C the medium was replaced with 2 ml of Ham's F-10 medium without serum. After another 24 h, medium was changed to fresh F-10 medium containing 0.1 μCi ml^{-1} ^{3}H-thymidine (specific radioactivity 2 Ci mmol^{-1}). The cultures were then given EGF (1.67 nM) and HSA (1 mg ml^{-1}) at the following times: EGF and HSA at 0 h (●); EGF at 0 h and HSA at 12 h (○); EGF and HSA at 12 h (△); EGF only at 0 h (▲). At the indicated times duplicate dishes were collected for autoradiography as described elsewhere[16,18].

Recent experiments by Livingstone *et al.*[14] indicate that insulin-dependent desensitisation of fat cells may be correlated with a decrease in the (low-affinity type of) insulin receptors. Similarly, desensitisation of rat Leydig cells to human chorionic gonadotropin *in vivo* was linked with a concomitant drop in hormone receptors[15]. These results differ from the present ones in some quantitative aspects, but together they suggest that modulation of receptor number may be a general mechanism for the regulation of cellular responsiveness to hormones.

This work was supported by grants from the Swedish Cancer Society (55 and 689), the Swedish MRC (4486 and 2309) and the University of Uppsala. We thank Miss A. Magnusson, Miss M. Pettersson and Miss Y. Öhgren for technical assistance, and Dr A. Karlsson for helpful discussions.

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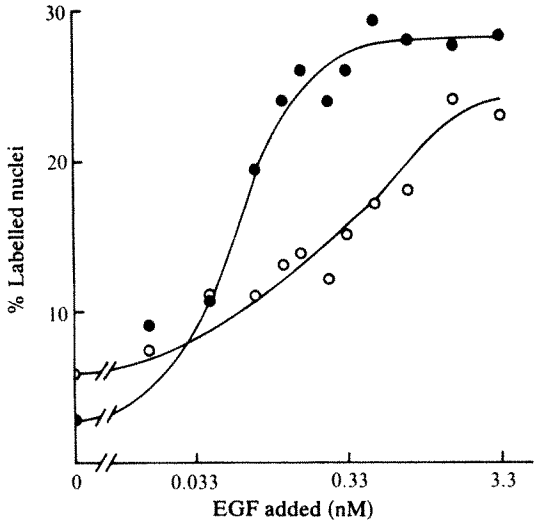


Fig. 3. Effect of EGF receptor down-regulation on the mitogenic response of human cultured glial cells to EGF. Glial cells were seeded as described in Fig. 1. After 24 h at 37 °C the medium was changed to Ham's F-10 without serum and the incubation continued for another 24 h. The cultures were then given 2 ml of fresh F-10 medium (●) or 2 ml of F-10 medium containing 167 nm EGF (○). After 22 h the cells were washed once with 2 ml of plain F-10 medium and five times with F-10 medium supplemented with HSA (1 mg ml^{-1}) and incubated in 2 ml of this medium containing various doses of EGF. After 24 h 0.4 μCi of ^{3}H-thymidine were added and incubation continued for another 60 min. Cultures were then collected for autoradiography[16,18]. Each point represents the average of duplicate dishes.

Adipokinetic hormone inhibits protein synthesis in *Locusta*

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The adipokinetic hormone (AKH) of the locust has been isolated, characterised and synthesised[1,2]. In this insect, its function is to mobilise diglycerides from the fat body to provide energy for sustained flight[3]. In addition, it modifies the metabolic activity of the flight muscles so as to oxidise fatty acids preferentially[4]. We report here another effect of AKH on locust metabolism, the direct inhibition of protein synthesis by the fat-body. This inhibitory activity may function in the locusts' overall adaptation to flight and it may also account for the presence of AKH both in larval locusts[5] and in other species which do not fly for prolonged periods[6].

There have been several studies of the neuroendocrine control of protein synthesis in the locust. Hill[7] has shown that electrocautery of the median neurosecretory cells (MNSC) of the brain brought about a diminution of protein synthesis. Osbourne *et al.*[8] reported that injection of homogenates of corpora cardiaca (CC) stimulated protein synthesis in adult female locusts. In larval locusts alterations in protein synthetic activity may be correlated with changes in ecdysone titre[9,10]. Because the prothoracic gland is under neuroendocrine control in the larval insect the neurosecretory control of protein synthesis may be indirect though the mode of control in the adult insect is not known. To investigate this problem further, we studied the role of the CC in protein synthetic activity. The locust CC presents an unusual opportunity for the study of insect neurohormones because it can be separated into 'storage' and 'glandular' lobes[13]. Activity was tested by injecting CC homogenates into 5-d-old fifth instar larvae together with 1 μCi ^{3}H-leucine (Amersham/Searle specific activity 50.6 Ci mmol^{-1}). Control insects received an equivalent

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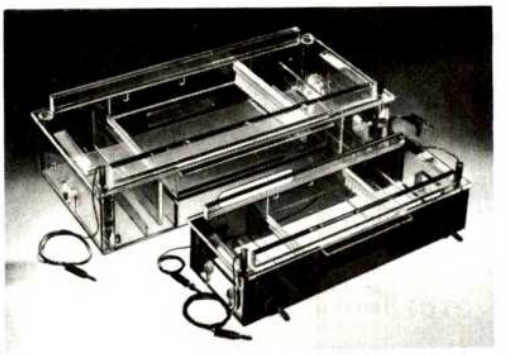
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injection containing a homogenate of the optic lobe of the brain. After 2 h the animals were bled through the cervical membrane and the incorporation of ^{3}H-leucine into haemolymph protein was determined[12]. When homogenates of whole cardiaca were injected (one gland in 10 μl saline) there was no consistent difference in incorporation of label into haemolymph protein between experimental and control insects.

However, when each lobe was homogenised separately, the injection of glandular lobe homogenates suppressed the incorporation of labelled amino acid into haemolymph protein by 24% compared with controls. A factor was found in extracts of the storage lobe that stimulated protein synthesis. The nature of this enhancement of protein synthesis will be reported elsewhere.

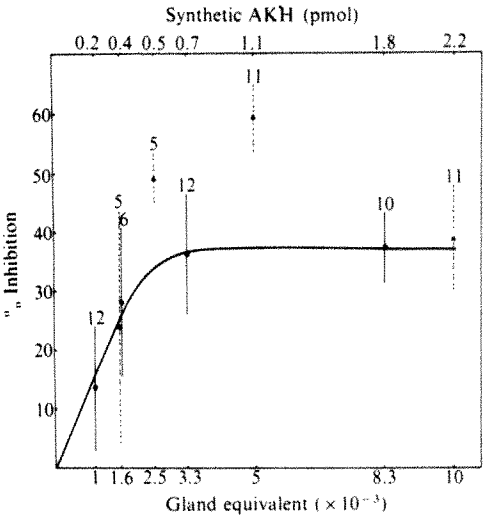


Fig. 1 The inhibition of *in vitro* protein synthesis by adult fatbodies incubated with homogenates of the glandular lobe of the CC (●), concentrations expressed in gland equivalents per 0.5 ml, and synthetic AKH(▲), concentrations expressed in pmol per 0.5 ml. Because the glandular lobes of the adult locust contain about 50 μg of AKH[1] the abscissae for synthetic hormone and for gland homogenates are approximately equivalent. To calculate percentage inhibition, incorporation of ^{3}H-leucine into half fatbodies incubated with gland homogenates or synthetic hormone was compared with that occurring in the contralateral half incubated with optic lobe homogenate. Bars represent twice the standard error. Integers above the bars indicate the number of replicate experiments. Synthetic AKH was a gift from Dr R. Downer.

The insects used in these experiments had a complete neuroendocrine system, and it was possible that the response detected was the result of the activation of one or more elements of their own endocrine system. To test this possibility the fatbody was dissected out from mature adult male locusts, divided longitudinally and each half was incubated *in vitro* in 0.5 ml Schneider's modified *Drosophila* medium (GIBCO) containing 1 μCi ^{3}H-leucine. One half received a homogenate of one pair of glandular lobes in 10 μl of locust saline; the other received 10 μl of a similar homogenate of optic lobe in locust saline. After 45 min at 36 °C, 50 μl of the incubation medium was removed and incubation continued. After 2 h a second 50-μl sample was taken and incubation was terminated. The medium was collected; the fatbody was washed quickly in saline and then homogenised in 100 μl saline. A 20-μl sample of the homogenate was assayed for total radioactivity. A second 20-μl sample was assayed for protein[14] and incorporation of radioactivity into protein[12] along with samples taken from the incubation medium. The incorporation of labelled amino acids into released protein was expressed as a function of protein in the fatbody[15]. For convenience the incorporation of labelled amino acid into proteins will be termed protein synthesis although the size of the amino acid pool was not determined. Because the rate of incorporation was not immediately affected by the gland homogenate, the degree of inhibition was determined by comparing the difference in incorporation of label by the control and treated fatbodies during the last 75 min of incubation. The homogenate of 1/100 glandular lobe suppressed protein synthesis by 38% (Fig. 1). A similar level of inhibition occurred whether fatbodies were taken from fifth instar larvae (5-d-old) or mature adult males. The relationship between concentration of gland extract and the level of inhibition of protein synthesis is shown in Fig. 1. This inhibition of incorporation of label followed a similar pattern in the protein of the fatbody to that of the protein released into the incubation medium. No significant difference in the amount of label in the free amino acid pool of the control and experimental fatbodies could be detected. Gel electrophoresis[16] of the medium after inhibition showed that incorporation of label into all proteins was equally affected by the gland homogenate.

Cheeseman *et al.*[19] have estimated that 0.007 gland pair equivalents are released *in vivo* in response to a short flight resulting in a 135% increase in haemolymph lipid. Because the blood volume of an adult male locust is approximately 360 μl (ref. 20), Fig. 1 shows that significant inhibition of protein synthesis occurs at concentrations of AKH likely to be found in the animal.

The inhibitory activity of the gland extract was stable to boiling for 15 min and to freezing at −12°C, although some activity was lost after both these treatments. The activity was destroyed when gland homogenates were digested with protease (Sigma) (1 mg ml^{-1} at *p*H 6.5 in 0.1 M phosphate buffer for 18 h). Electrophoresis of the homogenates in 5% polyacrylamide gels showed the activity to migrate close to the ion front. These data suggested similarities between the inhibitory factor and locust AKH which is the only hormone demonstrated in the glandular lobe of the CC so far[17]. The relationship between concentration of synthetic AKH and suppression of protein synthesis *in vitro* by adult fatbody is shown in Fig. 1. This relationship leads us to believe that the inhibitory activity of glandular lobe homogenates is due to the presence of AKH.

Mayer and Candy[18] reported that injection of CC extracts into locusts brought about mobilisation of fatbody lipids which was similar to that during flight. It is generally accepted that AKH promotes efficient flight metabolism. It is possible that the suppression of protein synthesis in the flying insect represents another example of such a metabolic switch. The inhibition of protein synthesis by AKH may be significant in circumstances other than flight. AKH is present in larval locusts in which it exerts little effect on lipid mobilisation[5] but a considerable effect on protein metabolism. It is possible that the inhibition of protein synthesis is the predominant action of AKH in larval locusts.

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A transposon, Tn732, encoding gentamicin/tobramycin resistance

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Gentamicin and tobramycin are important antibiotics in the treatment of hospital infections because of their activity against a wide range of bacterial genera. With their increasing use, bacteria resistant to these drugs have appeared, the resistance being frequently plasmid determined[1-4]. The resistance genes determine various enzymes that modify and inactivate the drugs[5] and there is association between particular gentamicin/tobramycin resistance genes and plasmids of particular groups[6,7], implying that acquisition of such a gene by any plasmid is a rare event[4]. We now report the identification of a transposon or 'jumping gene' encoding the gentamicin/tobramycin adenylylating enzyme, ANT(2″), on a plasmid of incompatiblity group FII (IncFII).

This plasmid, pHH1313b, confers resistance to ampicillin, tetracycline, chloramphenicol, gentamicin and tobramycin (Table 1) and was identified during a study of the spread of gentamicin/tobramycin resistant *Klebsiella* in a Toronto hospital[9]. The gentamicin/tobramycin resistance is conferred by the adenylylating enzyme ANT(2″)[7]. It is a non-conjugative plasmid, and to look for transposition of gentamicin/tobramycin resistance, it was mobilised into the *Escherichia coli* K12 *rec*A strain, PB1455 (*thr*$^-$*leu*$^-$*thi*$^-$) using the plasmid R751 (Table 1). Another IncFII plasmid, R1, conferring resistance to ampicillin, kanamycin, sulphonamide, chloramphenicol and streptomycin (Table 1) was transferred into PB1455 (pHH1313b.R751) by conjugation. To look for transposition of gentamicin/tobramycin resistance to R1 we selected for the transfer of gentamicin/tobramycin resistance into the K12 strain J62-2 (*pro*$^-$*his*$^-$*trp*$^-$*rif*^{R}). All of the gentamicin/tobramycin resistant transconjugants obtained were screened for the other resistance markers of pHH1313b, R1 and R751. Ninety per cent of them carried the resistances of pHH1313b and R751 and had resulted from the mobilisation of pHH1313b into J62-2 by R751. However, 10% carried the resistances of R1 plus gentamicin/tobramycin resistance. Some of these colonies were lysed on top of 0.7% agarose vertical slab gels using the method of Eckhardt[10] in order to show their plasmid content. In each case one plasmid of molecular weight (MW) 70×10^6 was seen (Fig. 1). Thus the transposition of gentamicin/tobramycin resistance to R1 resulted in an increase in MW of 7.5×10^6. We have designated this transposed sequence Tn732. The frequency of its transposition to R1 was 1 in 10^6 which is low, compared with other transposons, although within the reported range[11].

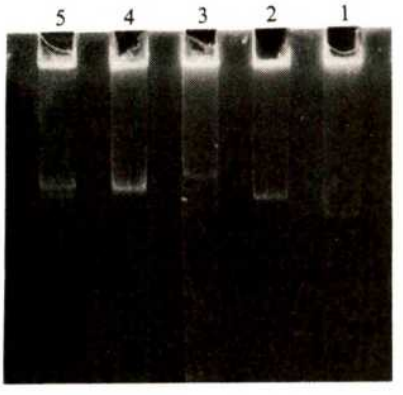


Fig. 1 Single colony lysates of R1 and R1 : Tn732. Colonies were lysed directly on top of a 0.7% agarose vertical slab gel as described by Eckhardt[10]. Standard plasmids of known MW were included. 1, R702, 46×10^6; 2, R1, 62.5×10^6; 3, RA1-1, 85×10^6; 4, R1 : Tn732, 70×10^6; 5, a colony of R1 : Tn732 + R1 lysed together in the same well. Gels were run for 6 h at a constant voltage of 170 V as described in ref. 4. MWs of standard plasmids were obtained from ref. 8.

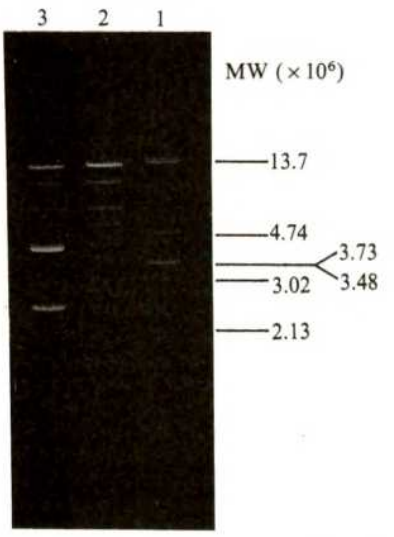


Fig. 2 *Eco*RI restriction enzyme analysis of R1 and R1 : Tn732. Purified plasmid DNA was isolated by a sarkosyl-cleared lysate method (K. Hardy, personal communication). Digestions were performed as described in ref. 4. Digested DNA was run on a 0.7% agarose horizontal submerged gel of dimension $20\times20\times0.4$ cm for 3 h at a constant current of 80 mA. 1, *Eco*RI digested λ DNA; 2, *Eco*RI digested R1 DNA; 3, *Eco*RI digested R1 : Tn732 DNA. Gels were calibrated using the known molecular weights of the λ *Eco*RI fragments[12] and the MWs of the R1 *Eco*RI fragments[13].

Purified DNA was isolated from R1 and from one of the clones of R1 : Tn732. The two plasmids were compared by digestion with the restriction enzyme *Eco*RI (Fig. 2). In this case Tn732 had inserted into R1 in such a way that the difference between R1 and R1 : Tn732 is the presence of two extra fragments with MW of 4.3×10^6 and 2.8×10^6 (Fig. 2). Further analysis of *Eco*RI digests using 2% agarose gels, which showed the smallest *Eco*RI fragments of R1 (ref. 13), confirmed that the only differences between R1 and R1 : Tn732 were the extra fragments in R1 : Tn732. It appears that Tn732 had inserted into an existing *Eco*RI site, duplicating the site and generating the extra fragments.

This is the first report of the identification of transposable gentamicin/tobramycin resistance. Transposons increase the chances of dissemination of resistance genes and it will be interesting to know how widespread Tn732 is among bacteria

Table 1 Plasmids used in this study

Plasmid	Resistances	MW	Inc group
pHH1313b	ApTcCmGmTm	62×10^6*	FII
R1	ApCmSmSuKm	62.5×10^6†	FII
R751	Tp	30×10^6†	P

Ap, ampicillin; Tc, tetracycline; Cm, chloramphenicol; Gm, gentamicin; Tm, tobramycin; Sm, streptomycin; Su, sulphonamide; Tp, trimethoprim; Km, kanamycin.

* M.E.N., unpublished observation.

† From ref. 8.

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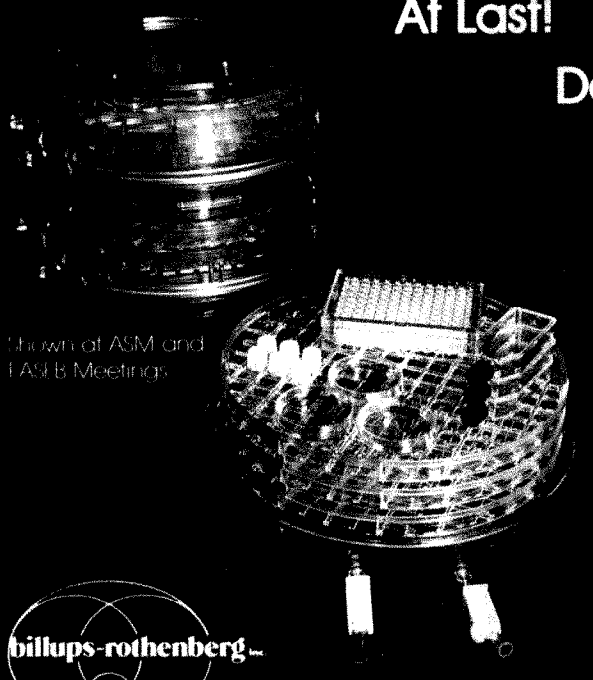
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and their plasmids. Work is in progress to characterise Tn732 further using restriction enzyme analysis. Once the finer structure of Tn732 is known we hope to use a radioactively labelled fragment of Tn732 in a filter hybridisation assay to show its spread elsewhere.

This investigation was supported by a grant from the MRC to N.D.

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Carboxy-terminal amino acid sequence of α-tubulin from porcine brain

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Tubulin is the main protein of microtubules, which are found in all eukaryotic cells. These structures participate in cell division, intracellular transport and secretion processes, ciliary and flagellar movement, morphogenesis and cell orientation. Tubulin is generally assumed to consist of two different chains, α and β, each of molecular weight about 55,000, which form a heterodimer in solution[1]. Recently, however, heterogeneity within each type of chain has been claimed on the basis of immunological non-identity[2], peptide mapping[3], isoelectric focusing and gel electrophoresis[4,5]. Another type of heterogeneity has been studied in more detail: a specific ligase binds one tyrosine residue to the carboxy-terminal residue of only a fraction of the tubulin α-chain[5–7]. The enzyme has been found in many tissues of various species[8,9], but the biological significance of the reaction is unknown. Elucidation of the primary structure should clarify whether a family of tubulins exists within one organism, and whether tubulin functions are regulated by post-translational modification. Furthermore, if a general principle underlies the various intracellular movements, one might expect sequence homologies between tubulin and muscle proteins. We have now established the sequence of the carboxy-terminal 78 amino acid residues in α-tubulin from porcine brain by Edman degradation of overlapping cyanogen bromide, tryptic and chymotryptic fragments. A terminal tyrosine was the only evidence for heterogeneity. The region is unusually acidic and not homologous to known proteins. Prediction of the secondary structure suggests a 'jack-in-the-box-like' structural change, dependent on the microenvironment.

We have purified tubulin from porcine brain by a modification of the methods used by Eipper[10] and by Ludueña *et al.*[1]. The 100,000g brain supernatant in 0.05 M sodium pyrophosphate buffer, pH 7.0, was incubated with 0.1 mM colchicine for 15 min at 37 °C before chromatography on DEAE-cellulose with a linear gradient of 0.1–0.3 M sodium chloride. Tubulin was identified by the fluorescence of its complex with colchicine[11]. The preparation was assayed for protein impurities by disc gel electrophoresis in the system of Yang and Criddle[12] using 8% gels. The gels were stained with Coomassie blue and scanned in a Vernon scanner. Only tubulin of more than 95% purity was processed further.

For separation of α- and β-chains the protein was reduced and alkylated with iodoacetic acid[13] and chromatographed on hydroxylapatite in 0.1% SDS with a linear gradient of 0.2–0.4 M sodium phosphate[14]. Fractions were assayed for purity by gel electrophoresis as above. Only α-chain of at least 95% purity was used for sequence determination.

The sequence of 78 residues at the carboxy terminus of tubulin α-chain is shown in Fig. 1. It must represent the terminal region because it contains the only tryptic peptide without a basic residue and the only cyanogen bromide piece devoid of a methionine-derived homoserine lactone. Furthermore, the terminal Glu corresponds to the glutamic acid previously determined to be the last amino acid in the α-chain[17].

The entire segment is devoid of proline and glutamine and has an acidic 'tail'. Residues 78–41 have a net charge of +4, are rich in alanine and in large side chains, whereas the last 40 positions have a net charge of −17, and are extremely rich in glutamic acid (40%) and glycine. It is by far the most acidic region of the α-chain and one of the most acidic sequences known.

Some heterogeneity could be expected from isoelectric focusing data. In this region of the chain, however, we found only one such case: the terminal tryptic peptide was isolated in two forms. About 85% of the material has nine Glu and one Tyr residues (composition Asp 1.88, Ser 1.08, Glu 8.80, Gly 3.99, Val 3.18, Tyr 1.07), whereas 15% shows nine Glu and two Tyr residues (Asp 2.03, Ser 1.17, Glu 8.89, Gly 4.09, Val 3.21, Tyr 1.60). The additional tyrosine is located in the C-terminal position. This corresponds to the observed post-translational binding of a tyrosine residue to the terminal glutamic acid by a tubulin-specific ligase[6]. Only about 45% of α-chain molecules can be tyrosylated[8]. This may be due to the presence of two different α-chains, although the C-terminal part has a homogeneous sequence. A cyanogen bromide fragment, comprising 26 residues almost identical to our C-terminal CNBr peptide, was isolated by Lu and Elzinga[18] from calf brain α-tubulin. These authors found one more glutamic acid at the C-terminus and discuss the possibility of a heterogeneity of Gly-Glu-Glu-Glu or Gly-Glu-Glu-Tyr. In our source, amino acid analyses of the two forms of tryptic peptide clearly show that tyrosine is added to a peptide of otherwise identical composition. Two other peptides,

```
 78                                  70
Arg-Ala-Val-Cys-Met-Leu-Ser-Asn-Thr-Thr-Ala-Ile-Ala-Glu-Ala-Trp-

          60                                                  50
Ala-Arg-Leu-Asp-His-Lys-Phe-Asp-Leu-Met-Tyr-Ala-Lys-Arg-Ala-Phe-

                              40
Val-His-Trp-Tyr-Val-Gly-Glu-Gly-Met-Glu-Glu-Gly-Glu-Phe-Ser-Glu-

 30                                          20
Ala-Arg-Glu-Asp-Met-Ala-Ala-Leu-Glu-Lys-Asp-Tyr-Glu-Glu-Val-Gly-

                    10                                        1
Val-Asp-Ser-Val-Glu-Gly-Glu-Gly-Glu-Glu-Glu-Gly-Glu-Glu(-Tyr)
```

Fig. 1 Amino acid sequence of the C-terminal region in α-tubulin. Residues are numbered starting at the carboxyl terminus. Batches of about 200 mg of α-chain were cleaved with trypsin, chymotrypsin and cyanogen bromide, respectively. Enzymatic digests were fractionated on Dowex 1X2 in volatile buffers and re-chromatographed on phosphocellulose or cellulose thin layer. Larger enzymatic fragments and the cyanogen bromide cleavage products were separated on columns of Sephadex G-50 Superfine (190 × 1.5 cm) in 8 M urea containing 0.1 M ammonium bicarbonate, and desalted on Sephadex G-10. Amino acid analyses, enzymatic digestions and sequence analyses by automated and manual methods were carried out as described previously[15], except that HPLC was used instead of gas chromatography for the identification of degraded phenylthiohydantoins[16] and assignment of side chain amides.

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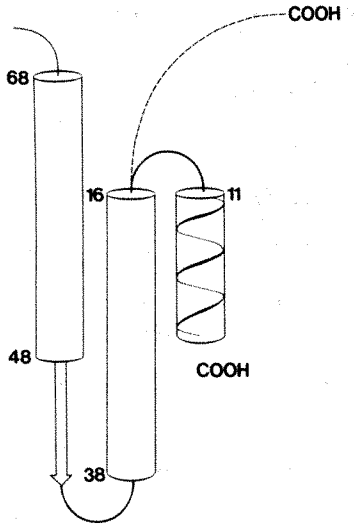


Fig. 2 Predicted secondary structure of the C-terminal region in α-tubulin. Residues 68–48, 38–16 and 11–1 are helical, position 47–42 represents a strand of a β-sheet and residues 41–38 and 15–12 are reverse turns. A jack-in-the-box transition of the labile terminal α-helix to a more extended structure is proposed to take place. The stability of each configuration depends on the microenvironment because of the many glutamic acid side chains, which may repel each other if fully dissociated. A C-terminal tyrosine increases the lability of an α-helix.

characterised but not ordered by Lu and Elzinga[18], match residues 62–53 and 37–26 in our sequence.

We could not detect any significant homologies to other known protein sequences, including bacterial flagellin, nucleotide and calcium binding proteins, actin and other muscle proteins.

The procedure of Chou and Fasman[19] was used to predict the secondary structure of this region (Fig. 2). It yields an α-helix at residues 68–48 (helix potential $P_\alpha = 1.20$ compared with potential for a β-sheet $P_\beta = 0.96$), a strand of a β-sheet at residues 47–42 ($P_\alpha = 1.00$, $P_\beta = 1.42$) which is connected by a reverse turn at positions 41–38 (turn potential $P_t = 1.12$, $P_\alpha = 1.03$, $P_\beta = 0.73$) to a second helix at positions 38–16 ($P_\alpha = 1.26$, $P_\beta = 0.69$) followed by another reverse turn at positions 15–12 ($P_t = 1.24$, $P_\alpha = 0.85$, $P_\beta = 0.93$). This clearcut situation becomes increasingly labile towards the C-terminus. Residues 11–1 have a helix potential ($P_\alpha = 1.21$, $P_\beta = 0.59$), but the many carboxyl groups of the glutamic acid residues, if fully dissociated, may repel each other, as polyglutamic acid loses its helical conformation at about *p*H 5.5 and turns into a random coil at higher *p*H[20]. Furthermore, the interspersed glycines create an underlying potential for turns and the helix potential is decreased by the addition of tyrosine.

We propose a jack-in-the-box-like structural change of the C-terminal residues from a helix to a more extended structure, depending on the microenvironment, for example, a tubulin dimer in solution or incorporated in a microtubule. Experimental support for two different states of the C-terminus of the α-chain comes from the finding by Thompson *et al.*[21] that the post-translationally added tyrosine is exchanged much more rapidly in intact microtubules than in the soluble dimer.

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Stomatal conductance correlates with photosynthetic capacity

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Previous studies on the physiology of stomata in higher plants suggest that stomata influence the rate of CO_2 fixation in leaf mesophyll tissue. We believe that an equally important stomatal function has not been fully recognised; that stomatal aperture is determined by the capacity of the mesophyll tissue to fix carbon. We altered the capacity of leaves to fix carbon by various means, and found invariably that the diffusive conductance of the epidermis to CO_2 transfer, g, (which mainly depends on the number and dimensions of the stomata) changes in nearly the same proportion as the rate of assimilation of CO_2. Thus, the intercellular concentration of CO_2 (c_i), calculated as $c_i = c_a - A/g$ (where c_a is ambient concentration of CO_2, A is assimilation rate of CO_2), tends to remain constant providing c_a is kept constant. We used routine techniques[1] to measure A and estimate g in leaves placed singly in chambers. Conductance takes account of CO_2 transfer through both stomata and leaf boundary layer, the conductance of the latter being 0.5 mol m^{-2} s^{-1}.

Figure 1 shows the effects of applications of 3-(3,4-dichlorophenyl)1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport, and abscisic acid (ABA), a hormone which decreases stomatal aperture, on assimilation rate and leaf conductance in cotton plants. When DCMU was applied, A and g declined in the same proportion, the slope of the line implying that c_i is constant at 220 μl l^{-1}. When ABA was applied, a curvilinear relationship between A and g was obtained. Its shape is consistent with the suppostion that A declined as a result of decrease in intercellular CO_2 concentration brought about by partial closure of the stomata. A similar contrast is shown in Fig. 2. The four points lying almost on a straight line represent A and g in *Eucalyptus pauciflora* at four different irradiances. The line does not extend through the origin and thus c_i was not quite constant in this instance. However, the change was only slight; c_i decreased from 257 to 243 μl l^{-1} with increasing irradiance. The curves in Fig. 2 were computed from measurements of the influence of c_i on A (c_i having been varied by varying c_a) and use of the equation above with c_a taken as 320 μl l^{-1}. They are estimates of the relationships between A and g that would be manifest at each irradiance if g were directly perturbed, as was done in the cotton plants by use of ABA. Figure 3 shows rate of assimilation and leaf conductance in plants of *Zea mays*. Differences between plants were due to differing levels of nitrogen nutrition, phosphorus nutrition and water stress. The points approximate a straight line passing through the origin the slope of which corresponds to $c_i = 100$ μl l^{-1}. The continuous curves show how a direct perturbation of g would have influenced A in four particular plants having differing levels of nitrogen nutrition; they were computed in the same manner as those in Fig. 2.

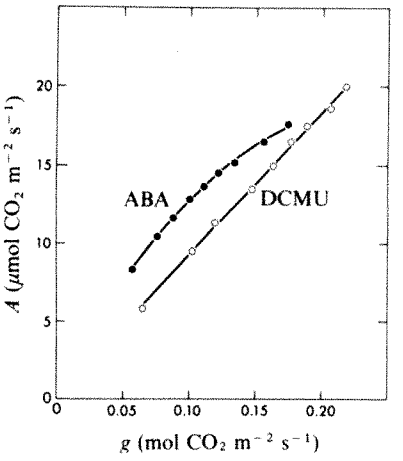


Fig. 1 Relationship between A and g in *Gossypium hirustum* as influenced by ABA, and DCMU. ABA (1×10^{-6} M) and DCMU (5×10^{-6} M) were applied in the water taken up by the detached, transpiring leaves. The different points represent differing periods of accumulation. Ambient CO_2 concentration, 320 μl l^{-1}, leaf temperature, 30 °C; leaf to air vapour pressure difference, 20 mbar.

The treatments which yield the straight line relationships in Figs 1–3 all influence the mechanisms of photosynthesis at the cellular level. The inhibitor DCMU reduces photosynthetic electron transport; increase in irradiance increases the potential rate of electron transport; nitrogen nutrition influences the amounts of chlorophyll and the activities of ribulose 1-5-bisphosphate (RuBP) carboxylase and phosphoenol pyruvate (PEP) carboxylase; and phosphorus availability affects photophosphorylation and the development of chloroplast lamellae[2]. Also, water stress, imposed slowly, caused a decline in both A and g such that intercellular CO_2 concentration remained essentially constant (Fig. 3), the implication being that stress directly affects photosynthetic carbon fixation. Diminution in the amount of soil water resulted in a 40% decrease in A and g but c_i remained constant at 100 μl l^{-1}. There are many other reports of observations of gas exchange which demonstrate the same proportional relationship between g and A; for example in *Phaseolus vulgaris*[3,4] and *Oryza sativa* leaves of various ages[5], in *P. vulgaris* and *Zea mays* plants grown at different irradiances during ontogeny[6], in *O. sativa* plants given different amounts of nitrogen[7], in leaves of *P. vulgaris*, *Z. mays* and *Imperata cylindrica* subjected to different irradiances[4], and in *Z. mays*[8], *Sorghum bicolor*[9] and *Eucalyptus socialis* seedlings[10] subjected to increasing water stress.

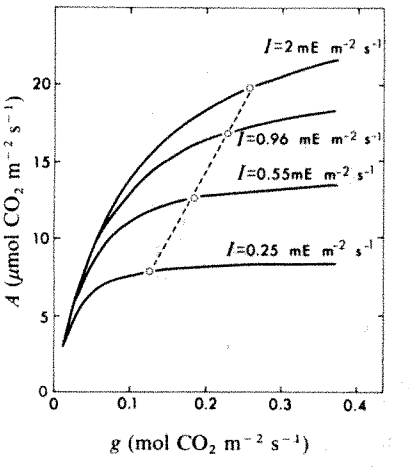


Fig. 2 Relationship between A and g in *Eucalyptus pauciflora*. A and g at four irradiances, I, during measurement with ambient CO_2 concentration = 320 μl l^{-1}, leaf temperature = 25 °C, leaf to air vapour pressure difference = 18 mbar. Curves represent the variation of A that would occur if g were independently perturbed; they are computed from measurements of the responses of A to changes in intercellular CO_2 concentration.

In eight C_3 plant species c_i was maintained at about 220 μl l^{-1} and in four C_4 species it was maintained at about 100 μl l^{-1}. These observations were made at an ambient CO_2 concentration of 320 μl l^{-1}, with a vapour pressure deficit of 20 mbar and with leaf temperatures near the optima for photosynthesis. The influence of leaf temperature on the relationship between g and A differed from that of the other factors so far considered. In general it was such that c_i increased at both low and high temperatures.

Linear relationships between A and g do not necessarily indicate that stomata control the rate of assimilation. When the source of variation was a treatment which directly affected stomata only, as with the application of ABA[11], the resultant variation in rate of assimilation (caused by the influence of conductance on c_i) was different (Fig. 1). Such curves also occur as a result of variation in ambient humidity[12,13] and rapid imposition of water stress[14,15].

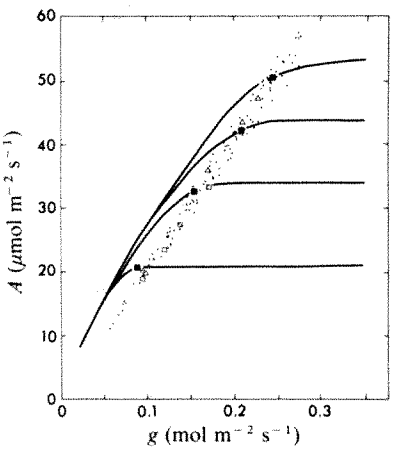


Fig. 3 Relationships between A and g in *Zea mays*, measured at irradiance of 2 mE m^{-2} s^{-1}, ambient CO_2 concentration, 320 μl l^{-1}, leaf temperature, 30 °C and leaf-air vapour pressure difference, 20 mbar: ·, plants having received different amounts of nitrogen and phosphorus nutrients; □, different stages in the recovery of a nitrogen deficient plant after nitrogen nutrient was given; △, different stages in the development of water stress in a plant from which water was withheld. The continuous curves represent the variations in A that would occur in four particular plants having differing photosynthesis capacities due to differing levels of nitrogen nutrient if g were independently perturbed. ■ Indicates actual values of A and g corresponding to c_a of 320 μl l^{-1}.

What, then, are the mechanisms responsible for the linear relationships in Figs 1–3? It is possible that stomata respond directly and independently to factors that influence photosynthetic carbon fixation. It is known that DCMU[16,17], irradiance[16,17] and water potential[16] influence stomatal aperture in isolated epidermal strips. Perhaps nitrogen and phosphorus also do so. Nevertheless, we think that the close correlation between A and g in a variety of conditions, including those associated with development suggests that there is a direct link between the two. The discovery[18] that the abaxial and adaxial patterns of stomatal response to light in a periclinal chimaera—between *Solanum pennellii* epidermis and *Lycopersicon esculentum* mesophyll—were almost identical to those in *L. esculentum*, and quite unlike those in *S. pennellii*, supports this notion.

A likely hypothesis is that stomata respond to changes in c_i so as to maintain it at a constant level by negative feedback. Raschke[16] and Raschke *et al.*[19] have attributed the variation in conductance with variation in irradiance to this mechanism. If the feedback loop maintains c_i constant at a preset value when irradiance is changed, then c_i should also remain constant when ambient CO_2 concentration is changed. Figure 4 illustrates a test of this last proposition. It shows that $\partial c_i/\partial c_a$ is about 0.7 in

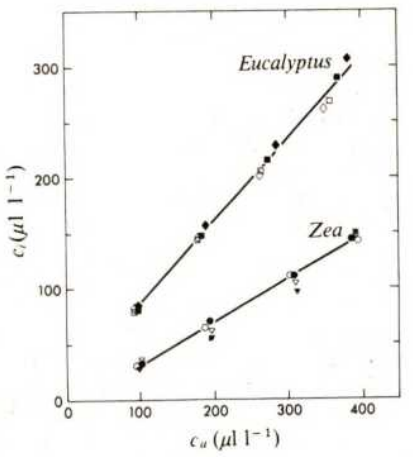


Fig. 4 Relationship of c_i to c_a, in *Eucalyptus pauciflora* at four irradiances during measurement: ◆, 0.25 mE m^{-2} s^{-1}; ■, 0.55 mE m^{-2} s^{-1}; □, 0.96 mE m^{-2} s^{-1}; ◇, 2.0 mE m^{-2} s^{-1}; and in *Zea mays* grown at four nitrogen levels. The *E. pauciflora* plant is that used to obtain the data shown in Fig. 2, and the *Z. mays* plants are those used to obtain the four continuous curves in Fig. 3.

Eucalyptus pauciflora and 0.4 in *Zea mays*. Apart from a report[4] concerning *Zea mays* at high c_a, we know of no instance when the coefficient has been found to be near zero[11]. Therefore negative feedback cannot entirely account for the near constancy of c_i when A is perturbed[1].

We consider that our evidence suggests an additional mechanism: that the stomata respond to another metabolite of photosynthesis in the leaf mesophyll tissue. There are several metabolites (such as ATP, NADPH and ribulose bisphosphate), the concentrations of which partially determine the capacity of the photosynthetic system to fix carbon, but decrease with an increase in the rate of carboxylation associated with an increase in intercellular CO_2 concentration. Their concentrations vary in a complex way with temperature. Our observations would be explained if stomatal aperture were related to the concentration of one of these compounds. This would be consistent with the tendency for stomata to close when the ambient concentration of CO_2 is increased. The suggestion also agrees with evidence of metabolite transport between mesophyll tissue and guard cells[20].

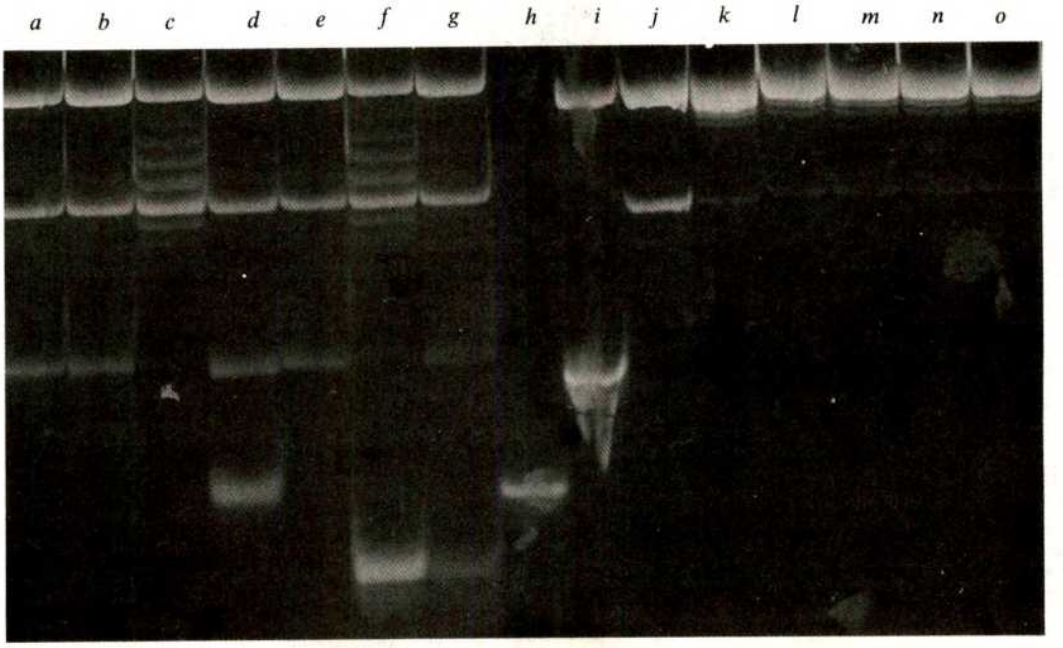
The extent to which stomatal responses modulate the rate of transpiration has been emphasised in the literature. However, it is clear that stomatal aperture is a compromise between the needs to conserve water and to maintain the rate of assimilation at a level dependent on the intrinsic capacity of the leaf mesophyll tissue to fix carbon. Our evidence indicates how closely conductance is influenced by that capacity and is relevant to theories of optimality in the regulation of gas exchange[21–23].

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Erratum

In the article 'Single strands induce recA protein to unwind duplex DNA for homologous pairing' by R. P. Cunningham *et al. Nature* **281,** 191–195, Fig 4 was reproduced such that some of the bands were lost. It is shown in a different form below.

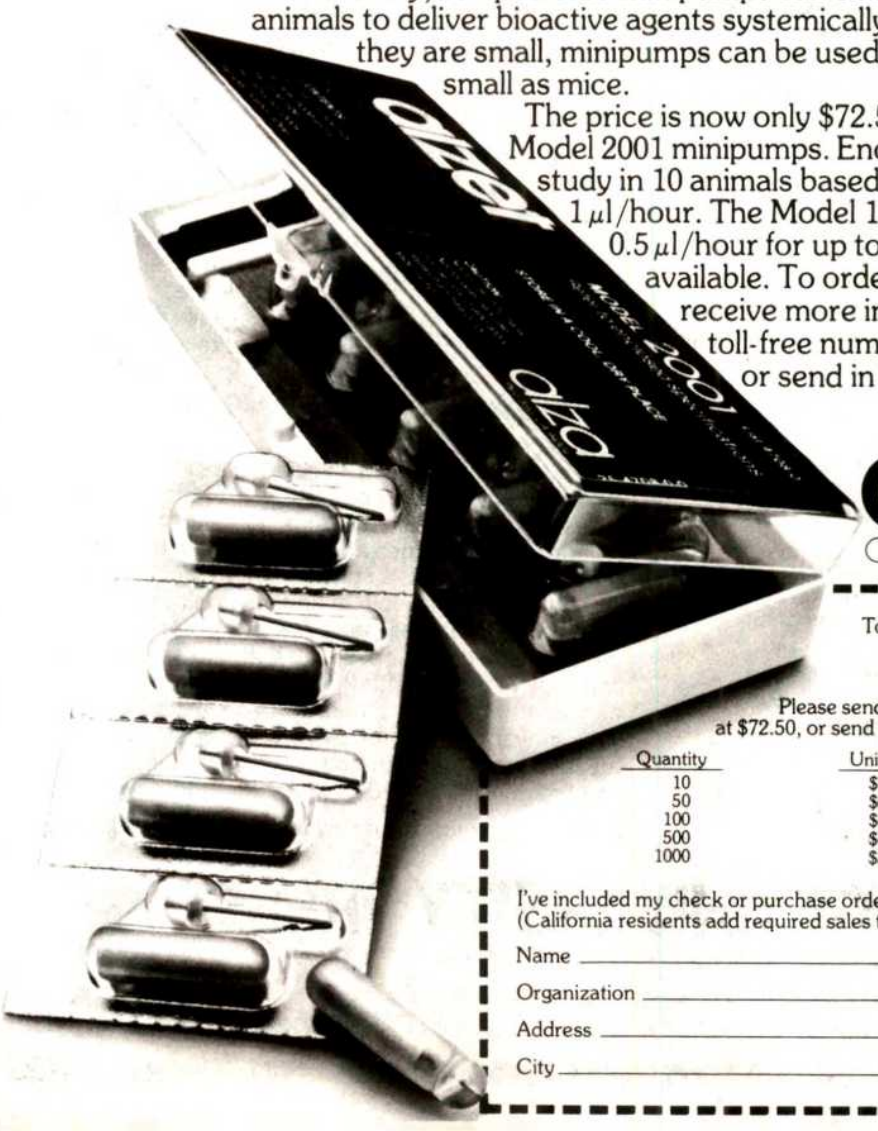


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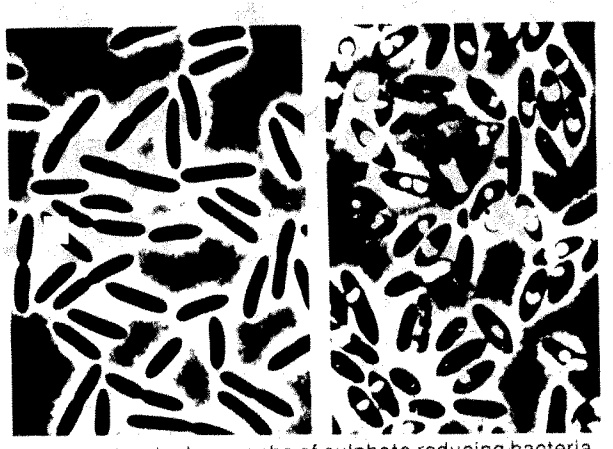
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reviews

Adapt or be damned

Eric Ashby

Uncertain Futures: Challenges for Decision-Makers. By R.U. Ayres. Pp.429. (Wiley-Interscience: New York and Chichester, UK, 1979.) £13.

To call futures 'uncertain' is an understatement. All that can be said with certainty about the future is that publishers still welcome books about it. Good luck to the publishers, for these books keep the debate going, and that is important. Dr Ayres makes useful contributions to this debate, though in style and content the book is in places exasperating. His contributions to the debate are that he gives priority to social and political issues and that he writes from practical experience as an industrial consultant whose living depends on the common sense of the judgements he makes; so his essay is comparatively free from the irresponsibilities of academic utopians and doomsters.

The preface is excellent. To forecast the future, Dr Ayres says, one has to examine not only technological issues, "but the trends in the culture — in society's political and social life, in . . . its people's attitudes, beliefs, and customs". Time after time, "it turns out to be social, political, ideological, legal, economic, and religious issues that stand in the way of technological solutions to human problems". He makes the distinction between projections — contingent statements which are valid provided all the 'if' clauses are clear — and predictions of what *will* happen in the future, most of which, he says, "are either disreputable or ambiguous". And, as Dr Ayres' examples show, most projections, though valid, are so weakened by the 'ifs' that they are precious little use to decision-makers. He backs up his argument with nice examples: thus IBM and Kodak lacked the foresight to invest in the infant technology of xerography, and a distinguished astronomer royal is reported to have said, one year before Sputnik I went up, that "space travel is utter bilge".

Dr Ayres' approach to the practice of forecasting is sober and systematic. First, the forecaster himself must be scrutinised. Is he a conservative who sees change as incremental projections from the present? Or a radical who sees change as cataclysmic discontinuities with the present? Does he create scenarios using as a tool a computer or his imagination? Is his approach clinical and objective or does he speak as a committed advocate? What is his implicit theory of history? Does he believe that leaders really lead? Or do they simply articulate what 'society' believes? He makes the important point to which mathematicians are now trying to apply catastrophe theory: namely that human institutions do not adjust to small pressures. The pressures build up — as the earth-stresses do before an earthquake — until a critical point is reached and there is an explosion; it happened in Lebanon, in Ulster, in the Southern States of America, in Rhodesia; and it is likely to happen over the energy shortage in the United States and the food shortage in India. (There is a wry similarity between the gas-guzzling Cadillac in America and the 100 million or so sacred cows in India: the one wasting oil resources, the other wasting food resources; the one a symbol of the religion of materialism, the other a symbol of an ancient oriental religion.)

After this admirable introduction, the book becomes disappointing. Dr Ayres makes a systematic appraisal of the present state of Mankind (he calls it 'measures of man'); man's values, his international relations, his prospects judged from the present levels of population and economics, his technology and the way it seems to be going, and the resources left in the world (and from the Sun) to carry Mankind into the next millennium. He uses familiar examples and draws on secondary sources (and some of them not very expert sources at that). To judge him by his own criteria for scrutiny, he is an incrementalist with misgivings about the prospects for incrementalism; a forecaster who prefers to use imagination rather than to rely on quantitative data; an analyst who tries hard to follow his own recipe (forecasters must be "indifferent to outcomes") but whose personal convictions keep breaking in. The bulk of the book records Dr Ayres' views on these 'measures of man' supported by telling anecdotes and some rather sketchy data. This is no bad thing; there are plenty of data to be found elsewhere, but the book has to be judged on the originality of its reflections rather than the comprehensiveness of its information.

I think it would be a fair summary of Dr Ayres' views to say that they are an articulation of what in Britain might be called sound CBI (Confederation of British Industry) doctrine. He has the courage to say some unpalatable things. He is apprehensive about the future of liberal democracies and he holds out little hope that Britain's brand of 'soft socialism' which stresses equity and freedom at the cost of efficiency, will even be able to preseve equity and freedom in the long run. He makes a list of wealth-creating values (motivation, positive attitude to work, integrity and personal honour, concern about the future) and describes how these are being eroded. (He might have added loyalty to the firm, to the institution, to the employer, for the erosion of these loyalties is the most obvious national danger.) He has some robust down-to-earth things to say about some of the current panaceas we talk about. Plans for rural development, for example, he thinks are impracticable, for the infrastructure necessary must be created by an urbanised society; and, with the prospect that transport will become more difficult as the oil shortage bites, there may be a revival of urban living. Among the attitudes which contribute to the growing malaise in western civilisation Dr Ayres selects two for special treatment: one is the resistance to innovation exemplified by a wide variety of practices, from the 'catastrophic' regulations which inhibit the production of new pharmaceutical drugs, to the attitude of some trade unions which (as he bluntly puts it) is to "increase the number of man-hours required to produce a unit of output, *at no decrease in wages*, as a device to create even more unnecessary jobs". The other attitude which Ayres criticises is the proliferation of what he calls "parasitic professions". Lawyers, in particular, get his goat: "the most parasitic single group in the United States . . . a unique ability to create demand for their own unproductive services". Health services, battening on Blue Cross, come in for similar, though milder, criticism. He challenges the medical 'ethics' which prevents doctors from competing by offering definite services for fixed prices, and he thinks fewer resources ought to be spent on the unproductive practice of keeping old people alive who suffer from degenerative diseases. Education is another social activity which, in Dr Ayres' opinion, is failing to equip the next generation to cope with its uncertain future. There is, he believes, a decline in quality of education due in part to unionisation among teachers ("the organization of effective teachers' unions has tended to reduce the average level of performance by the teachers . . ."), in part to time wasted looking at TV, and in part due to the use by children

of minicalculators.

When Dr Ayres talks about technology he is on firm ground, but his knowledge of economics and political science unfortunately does not match his admirable determination to give these subjects a higher priority than technology as determinants of the future. However, towards the end of the book, in the last 75 pages, he no longer comments on familiar facts; instead he gives free rein to his imagination. He describes, in the form of fictitious brief press extracts, the way in which a world energy crisis might develop in the west, or a food crisis in the Third World. As a scenario for the possible political response to an oil shortage between 1981 and 1985, I found the 18 pages of 'press cuttings' far more convincing than any sophisticated computer print-out. Ayres cites precedents from past history for some of his speculations about future history, and he presents the reader with a spine-chilling but quite credible series of social reactions which might follow another embargo of oil from the Middle East.

Forecasters, writes Dr Ayres in his preface, "must be independent-minded and indifferent to outcomes". He may have started to write this book as a true-blue forecaster. He ends the book as an advocate. Perhaps that is the moral to be got from the book: that the intervening chapters dispel indifference. It is as well they should, for what emerges from this book, despite its superficialities and its flimsy supporting evidence, is not superficial at all. It is that democratic institutions, "never deeply rooted in most countries, have already proved to be pretty much incapable of grappling with problems for which all the solutions are painful". This, in my view, is a more imminent and serious danger than the danger of nuclear war. And an insidious danger, too, for there is no external enemy to be defeated: the enemy is within and invisible. President Carter asked the American people to regard the energy crisis as the "moral equivalent of war". But this is to ask a nation to adapt itself *in anticipation* of an environmental change, not — as happens with organisms other than man — as a *response* to the change after it has occurred. The American people have not responded. The sixty four dollar question is: will communities of people respond in anticipation in a way that communities of animals would not? We may not have to wait long now for an answer. □

Lord Ashby is Chancellor of the Queen's University, Belfast, and Fellow of Clare College, Cambridge, UK.

Perceptual phenomena

Handbook of Sensory Physiology. Vol. VIII: *Perception*. Edited by R. Held, H.W. Leibowitz and H. L. Teuber. (Springer: Berlin, Heidelberg and New York, 1978.) DM240; $120.

OUR knowledge of perception has advanced in bursts. The mid-nineteenth century saw the inauguration of psychophysics, the doctrine of specific nerve energies, the foundation of colour vision, the discovery of the horopter, and the remarkable insights of Helmholtz. Between 1912 and the early 1930s, the discoveries of the Gestalt psychologists posed a whole new set of problems.

After a period of comparative quiescence, the modern era began in the mid-1950s. Since then, thanks to the development of many new techniques and the ingenious refinement of old ones, a wealth of new phenomena has been uncovered. The use of microelectrodes to record from single cells has revealed much about the amazingly systematic wiring that underlies the early stages of visual processing, and has made it possible to explain at least some psychophysical results in terms of known neurophysiological mechanisms. Thus, the dispute between Helmholtz and Hering about whether colour vision was achieved by a three colour system or by two pairs of opponent colours has been resolved: in the retina there are three different cone pigments, but more centrally hue is recoded into two mechanisms each based on opponent colours. Many other psychophysical phenomena, for example, those connected with visual acuity, stereoscopic vision and the perception of motion, also fit in with recent physiological findings, though their detailed interpretation is often still in dispute. We are, for example, still uncertain of the exact physiological correlates of the after-effects of prolonged inspection of motion or of a tilted line: some argue for a passive process of neuronal adaptation, others for a more active inhibitory mechanism.

Moreover, much recent work has served only to emphasise the extreme complexity and subtlety of perceptual mechanisms without necessarily clarifying them. In speech perception we have little idea of how phonemes are extracted, though we do know that there are no separate segments of the speech sound wave that correspond in a one-to-one fashion with individual phonemes. Despite the mass of work undertaken using new physiological and psychological techniques to investigate perception in infants and young animals, we do not understand the subtle interplay between maturation and experience. Nor is there any secure explanation of visual masking — the obliteration of one stimulus by a second presented several milliseconds later.

Perhaps even more dispiriting is the fact that many perceptual phenomena discovered long ago still await explanation: our understanding of the traditional visual illusions is no more secure today than at the end of the last century; nor do we know any more about the mechanisms that enable us to see an object as of the size it really is, despite the variations in the retinal size caused by changes in distance.

Only a man with the bravery and erudition of Hans-Lukas Teuber could have undertaken the task of editing a volume reviewing all the disparate results and theories that have emerged in recent years. He died before he had finished the job and it was left to the two co-editors whom he had co-opted to complete it. The finished product is worthy of his erudition. I can vividly recall him as he stood confronting one of his graduate students and saying in consternation, with that characteristic raise of his bushy eyebrows, "You mean you've never *heard* of Imaizikie?". The publication of Volume VIII of the *Handbook of Sensory Physiology* removes the wretched graduate student's last excuse for such ignorance. It provides a comprehensive source of references on the topics covered, but I feel it would not have met Luke Teuber's exacting standards in other ways. With the exception of his own contribution, the writing is for the most part pedestrian and fails to convey any sense of enthusiasm or excitement. Too many of the authors concentrate on listing facts or on attempting to resolve the many disagreements between recent results: they fail to place the findings they record in the context of what perception is all about. There are, of course, exceptions such as A. M. Liberman and M. Studdert-Kennedy who write on phonetic perception.

The vast number of phenomena recorded in this *Handbook* would have been easier to assimilate had more of the authors asked themselves what light the findings throw on the way in which perceptual tasks are carried out. Indeed, the one approach to the subject that is almost wholly missing from this volume is the theoretical study of precisely this question, that is, work on computational models of perception. Such research has revealed the role of the mechanisms discovered by microelectrode work: they provide a description of the retinal image that can in turn be manipulated to yield a representation of the external world.

In short, this *Handbook*, despite the solemnity of its approach, fails to make much sense of perception, and the boring parade of findings it presents is not worth the outrageous price asked by the publishers.

Stuart Sutherland

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex, UK.

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Behavioural neurobiology handbook

Handbook of Behavioral Neurobiology. Vol.2: *Neurophsychology.* Pp.566. Edited by M.S. Gazzaniga. (Plenum: New York, 1979.) £35.

THE general public expect from academic psychologists useful, and preferably unexpected and striking, insights into the human condition. They are interested in the psychology of conscious experience rather than in the minutiae of behaviour and performance usually discussed in academic lecture theatres. It is arguable that while they remain concerned only with human performance academic psychologists can seldom be interesting and never surprising. At best they can clarify the precise nature of limitations of which we have all been aware all our lives, and they make their greatest contribution only by suggesting models for central nervous system function which explain why we have these particular limitations and not others.

In contrast neuropsychologists, who study changes in function after brain damage or central nervous system disease, have far more surprising and vivid stories to tell. Their recent discoveries concern the very texture of daily experience and sometimes offer scary glimpses into the darkness within. Their work raises questions about ourselves and our experience which could hardly have occurred to us in any other way. Until Weiskrantz's recent demonstrations it would have appeared merely a far-fetched philosophical exercise, based on an elementary semantic misconception, to enquire whether any alert and articulate human being could, deliberately on request, make accurate discriminations between patterns which he cannot *consciously* report seeing. It is also hardly likely that we should have asked, as Le Doux, Wilson and Gazzaniga do in this book, not only whether the two hemispheres of the brain have different and independent functions but also whether, when separated surgically, they may have some degree of independent conscious experience while they share, *but independently interpret in different ways,* a commonly experienced shift in emotional tone controlled by lower brain centres communicating with both independently.

This second volume of the *'Handbook of Behavioral Neurobiology'*, edited by Gazzaniga, provokes many such startling questions. It also illustrates many of the conceptual and practical difficulties which beset this fascinating field of new knowledge about ourselves.

The title *Handbook* raises expectations of a detailed comprehensive reference work. Few of the contributors seem willing to attempt this useful task. Exceptions are the chapter on aphasias by Hecaen, on the frontal lobes by Jonander and Gazzaniga, on thalamic mechanisms in language by Brown, and a useful chapter on long term consequences of central lesions by Newcombe and Ratcliff.

Only a concise chapter on assessment of cognitive deficits in brain-injured patients by Goodglass and Kaplan literally meets the promise of the book's title by providing a handy guide to a complex applied literature for novice practitioners. This chapter, inadvertently, highlights a crucial difficulty currently obstructing progress in neurophysiology. Most tests of function currently used by neurophysiologists and neurosurgeons are crude, pragmatic survivals from wards and consulting rooms fifty or a hundred years ago. They have survived because they are rapidly administered indices which have been found, by experience, to very roughly categorise particular kinds of deficit when and if they are supplemented by the (often brilliant) intuitions of clinicians who, ritualistically, still administer them. A human experimental psychologist, accustomed to constructing models for normal performance at similar tasks, is at once struck by the fact that these particular tests involve such a complex muddle of interacting performance indices that it is quite impossible to say what are the precise functional deficits in performance any of them can demonstrate. However well they may serve as *ad hoc* diagnostic tools (not too well, reading between the lines of Goodglass and Kaplan's lucid chapter) they as yet give us little insight into the fascinating changes in function which actually accompany particular brain lesions.

The obvious solution is for human, experimental psychologist, knowledgeable about specific indices of performance, to interest themselves in the problem of defining precisely how performance changes when brain lesions occur. Morris Moscovitch's chapter 17 (''Information Processing and the Cerebral Hemispheres'') is the nearest attempt to this desirable end. As we see from the bulk of this useful book even crude and conceptually muddled tests of residual function do work in practical diagnosis. The crucial question is why they work, when they work (that is precisely what are the deficits which each of them actually exposes), and whether better, more clearly understood and more efficient diagnostic procedures cannot be developed from the considerable expertise built up by human experimental psychologists. From this point of view Moscovitch's chapter is disappointing. He describes only his own work. He is not concerned with questions of diagnosis but with the relationship of abstract models of human information processing to differences in hemisphere function. This would be interesting if the experimental techniques he uses gave the results which he supposes that they do. In particular the results illustrated in Fig. 1b and Fig. 2 on pages 386-388 and Fig. 5a and b on page 389 may have a number of different interpretations other then those which he places upon them.

For me this is a very exciting book indeed. If the excitement is flawed by impatience I am sorry. But the limitations to further progress are very distressing to anyone who avidly reads chapters 1-4, 7, 9-12, 14, 15 and most particularly 17 for the *ideas.* This is a book which is a key to our current conceptualisations of what we are, and to how the thin bright film of our experience covers the complex darkness within us, out of range of our introspection but perhaps within the grasp of clever and accurate experiments. The lack of experiments is galling, but I would wish the pleasure I have had reading this book to be as widely shared as possible by other scientists of many different educations and fields.

Patrick Rabbitt

Patrick Rabbitt is Lecturer in Experimental Psychology at the University of Oxford, UK.

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Permutation symmetry in chemical and physical problems

Molecular Symmetry and Spectroscopy. By P.R. Bunker. Pp. 424. (Academic: New York, 1979.) $33.

THIS volume is the first comprehensive treatment of the use of permutation symmetry in molecules, that is, in chemical and physical problems. As such, it will be widely welcomed, and fills a void which has long plagued those who had a need for this material. Fortunately, the writing in this volume is extremely lucid.

The volume begins with a clear definition of permutation operations and groups, then adds the (space) inversion operation to lead to the complete nuclear permutation inversion group (CNPI). The author next leads the reader through the more conventional application of group theory to molecules, with, however, a quite unconventional set of emphases. This reviewer feels that persons previously unfamiliar with chemical applications of group theory would have considerable difficulty with the treatment given. Fortunately, however, one may assume today (and it seems that the author has done so, although he claims the contrary) that anyone interested in the subject matter of this book has previously attained a working knowledge of molecular point groups and their use in spectroscopic problems. The emphasis in the appropriate chapters of this volume lies with the symmetry properties of the Hamiltonian and of its eigenfunctions, a much more fundamental approach than that commonly pursued. After these preliminaries are dealt with in 8 chapters the real meat of the volume follows: the definition of the molecular symmetry group on the basis of permutation and space inversion and its applications, primarily to problems of molecular spectroscopy and to non-rigid molecules.

It is to be welcomed that the author has been very careful and explicit in his definitions. Thus, the emphasis on the distinction between space and molecule fixed coordinate systems has led to a clean and unambiguous distinction of the two inversion operations, E^* and i, which have been far from clear in much of the previous literature. Similarly the very careful phrasing of the conditions of feasibility, although they may at times seem redundant, make a significant contribution to the understanding of the subject matter.

The book is extensively illustrated with well-drawn figures, essential when discussing symmetry operations and their effect. The author has attempted, and it seems successfully, to illustrate each theoretical concept he introduces immediately with appropriate examples. For further emphasis, throughout the volume he has provided problems to illustrate the principles, and then given detailed solutions.

Although this book addresses itself to a relatively restricted audience — spectroscopists, and quantum mechanicians interested in classificaion of excited states — it may be expected to rapidly become an essential tool to this audience. It is further to be hoped that the volume will help to make more scientists aware of permutation symmetry and thereby provide new insights into other phenomena.

H.H. Jaffe

H.H. Jaffe is Professor of Chemistry at the University of Cincinnati, Ohio.

Electron microscopy in chemical investigations

The Chemical Applications of Transmission Electron Microscopy. By J. R. Fryer. Pp.286. (Academic: London and New York, 1979.) £18; $38.

THE electron microscope finds application in almost all branches of science and technology. Over the years there have appeared many books describing its uses in biology, medicine, metallurgy and materials science, but we have had to wait until now for a treatment of its role in chemical research. The reasons for such a lack are not far to seek. Chemistry is rarely as interested in morphology (except in polymers) as are biology and metallurgy for differing purposes. Its problems so often involve knowledge at the atomic and molecular level, whether of structure or function. In recent years, however, the instrument has found increasing application in surface chemistry (catalysts and thin films), crystal chemistry (defects, non-stoichiometry and phase changes), solution chemistry (clay minerals, polymers) and in chemical kinetics (solid-state reactions, intercalation).

Progress in these new fields has now been brought together and critically presented by an active worker. Description of the various applications is preceded by four chapters which set out the physical principles and operational techniques. The microscope itself is dealt with briefly, fuller accounts being readily available. Greater attention is paid to the interaction of electrons with the specimen, including radiation damage, and to the mechanism of image formation and contrast. A separate chapter is devoted to the special conditions of high resolution microscopy, where phase contrast and lattice imaging are principally involved. The design of ancillary apparatus, with particular reference to special stages, and the various methods of specimen preparation, are also surveyed.

The meat of the book, however, is comprised in the later four chapters describing the use of electron microscopy in a variety of chemical investigations. Not unnaturally, greatest space is accorded to catalysts and their reactions, to the structure of crystals (especially graphite) and to molecular crystals and radiation damage to them, all subjects in which the author has made important contributions. These chapters are very fully illustrated with micrographs, not always well reproduced in my copy of the book, and line diagrams. Polymers and proteins receive less attention here; they are adequately covered elsewhere in the literature. In spite of the blurb on the cover, there is very little on the use of the electron microscope in biochemistry and materials science, but after all Dr Fryer did not set out to write an encyclopaedia of its applications.

He has done very well within this restricted plan, and the book can be highly recommended to research chemists who wish to know what this comparatively new technique could do for them. Two reservations must be stated, however. First, the references are not so completely up-to-date as could be desired. Apart from publications from his own laboratory, there are only three or four post-1975 references. The consequent omission of a great deal of recent research, especially at high resolution, is much to be regretted and is probably ascribable to delays in printing and binding, which seem to be on the increase. My second reservation concerns the price. Little more than a decade ago we could expect a well produced scientific book to cost about £1 per 100 pages. The price of this volume works out at well over six times as much, with some sacrifice in quality. Though this may be of the same order as the increase in price of a motor-car, it outstrips the rise in chemists' salaries. Sadly, few of them will be able to afford Dr Fryer's excellent monograph, but I urge librarians in spite of equally restricted resources to get it for their shelves.

V. E. Cosslett

V. E. Cosslett is Emeritus Reader in Electron Physics in the Cavendish Laboratory, University of Cambridge, UK.

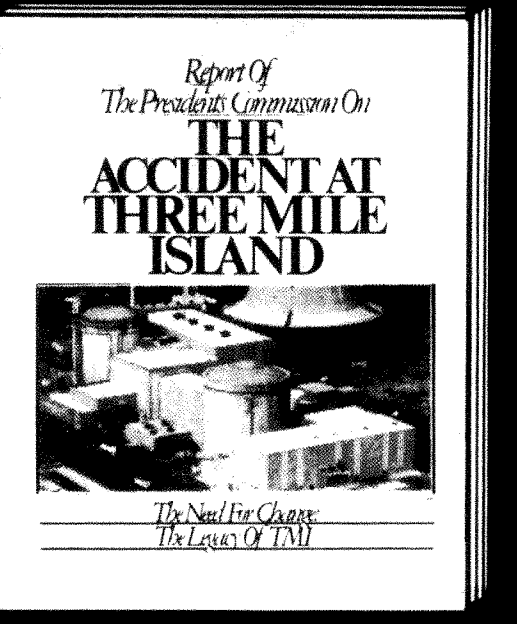
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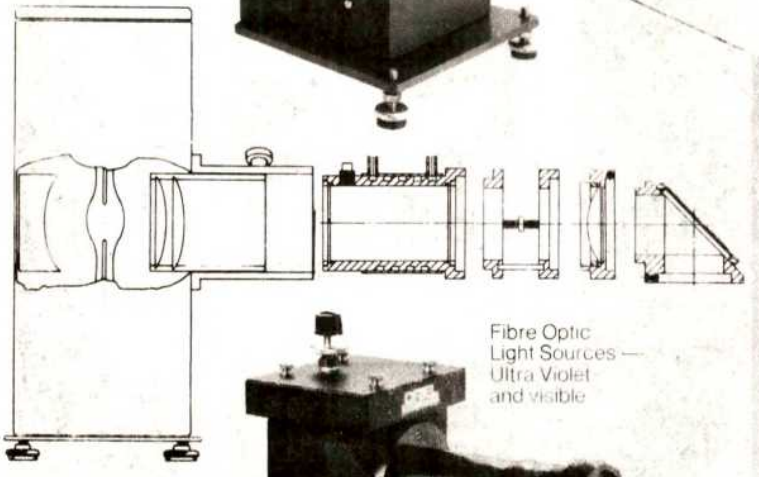
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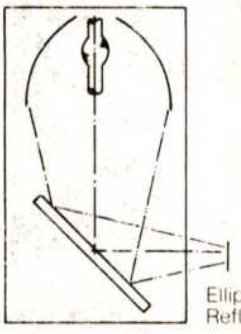


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new products: lab instrumentation

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A feature on water purification and analysis will appear in the 13 December issue.

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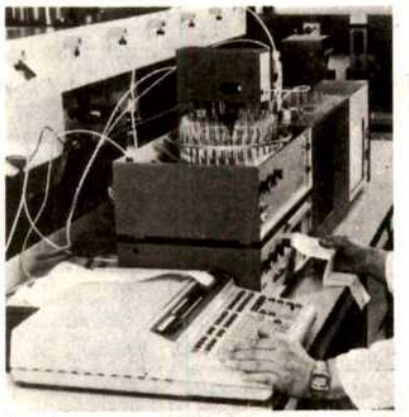
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A table computer in use with the Schott-Geräte viscosity measuring system

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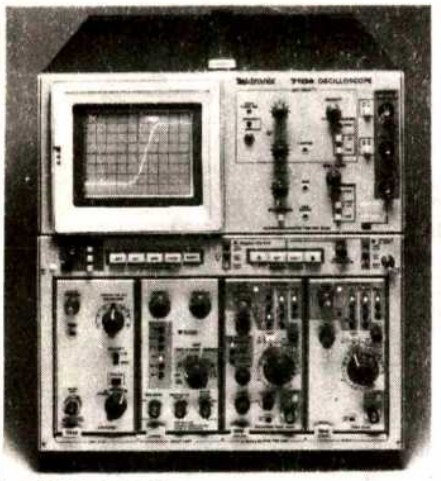
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LIQUID CHROMATOGRAPHY
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BIOMEDICAL RESEARCH

Bioanalytical Systems offers complete liquid chromatographs specifically designed for the biomedical research community. Modular liquid chromatographs are ideal for trace determinations of biochemically significant compounds such as catecholamines, indoles, sulfhydryls, amino acids, and PTH amino acid derivatives. Systems based on reverse phase chromatography and electrochemical or uv absorption detection are available in several flexible configurations.

Send for details . . .

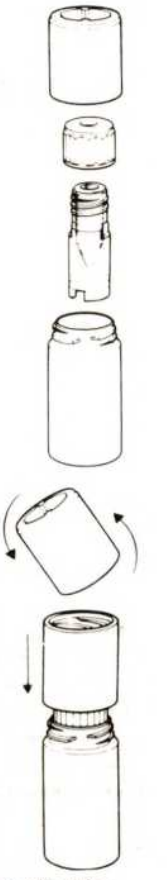
bas *BIOANALYTICAL SYSTEMS INC.*
Purdue Research Park • West Lafayette, IN 47906 • (317) 463-2505

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Using Iodine-125?

New Microvial*

- **reduced exposure**
- **reduced contamination risk**



Reduced exposure

The new packaging consists of 2 parts

- An outer plastic container incorporating a steel shield
- An inner plastic vial with a conical glass insert containing the iodide solution

Reduced contamination risk

The cap of the plastic outer container is keyed so that it can be used to unscrew the cap of the inner vial — no need to handle this vial from start to finish.

*patent applied for

The Radiochemical Centre Amersham

Full information is available on request
The Radiochemical Centre Limited, Amersham, England. Telephone: 024 04 4444
In the U.S.A. and Canada: Amersham Corporation, Illinois 60005. Telephone: 312 364 7100 and 800 323 9750 (Tollfree)
In W. Germany: Amersham Buchler GmbH & Co KG, Braunschweig. Telephone: 05307 4691

Circle No. 23 on Reader Enquiry Card.

SCHOEFFEL INSTRUMENTS

A new completely automated HPLC system was recently introduced by the Schoeffel Instrument Division of Kratos. The system utilises the new KLIC 1 interactive controller to control all the system elements which include the Schoeffel Series 250 chromatograph and detector. The extent of automation in the system makes it ideal for unattended operation, even overnight. With a 1,000-step programmable memory, the KLIC 1 is capable of control over gradient, flow, wavelength, and injection programming. Additionally, with up to 32 input ports, the KLIC 1 can observe and maintain the proper action of external system elements such as fraction collectors, auto-injectors, and pumps. This two-way, interactive feedback control permits automatic inprocess 'servicing' of malfunctioning components. The interactive feedback control can, for instance, sense a bubble in the pump head and automatically step-up the flow rate to flush it out, or, observing that a fraction collector has failed to advance, repeat the advance instruction until the proper action is executed.

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TRACOR NORTHERN, INC.

The Tracor Northern TN-4500 computer system is capable of true Foreground/Background operation utilising the popular RT-11 operating system. This permits routine PHA operation in the Foreground simultaneous with FORTRAN IV programming in the Background. Up to eight independent, multiport data memories support separates ADCs and MCS interfaces. Performance is further optimised through the use of a PDP-11/34 (with up to 118K memory) which provides the fast CPU necessary for efficient quantitative analysis. Dual density floppy disks provide program storage but for greater mass storage capacity.

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SPATIAL DATA SYSTEMS

The EyeCom II picture digitiser and display system from Spatial Data Systems is a complete image input and display device for quantifying the brightness variations in a scene or image. Using the EyeCom II in conjunction with most minicomputers will yield information on variations in image brightness and position, separation of image features, contrast enhancement of image differences, and sizing image features.

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MATEC INC

A gating modulator for use with a separate cw r.f. source in studies of nuclear resonance, ultrasonics, or any research area requiring high power, coherent r.f pulses is available from Matec, Inc. of Warwick, Rhode Island. The Matec Model 5100 is a mainframe gating modulator that produces single or double pulse sequences of high power, coherent r.f., when used with any Matec 500 Series r.f. amplifier plug-in. The circuitry keeps r.f. pulses coherent and eliminates time jitter of pulse widths and pulse separation.

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BRYANS

Series BS8000 microprocessor-based data-acquisition/manipulation recorders from Bryans are designed as alternatives to conventional X-Y recorders, strip-chart recorders, oscilloscopes, microcomputers or minicomputer systems. Featuring microprocessor-based data acquisition and manipulation, the Bryans Series BS8000 can have up to eight analogue input channels, each with a differential input. Matching software packages are available for: liquid chromatography, spectroscopy, battery research, thermal gravimetric analysis, thermal mass analysis, and mechanical engineering applications. Each model in the Series BS8000 range provides the following mathematical functions, to 16-bit precision: add, subtract, multiply, divide, average any two curves (500 points each) point by point, differentiate, integrate, logarithm, reciprocal, normalise, and smooth any curve (500 points) point by point. Plotting functions are also incorporated: offset, scale, expand, compress and shift. Real-time plotting is standard, unless the input signal is too fast, in which case the signal is stored and recorded automatically by the plotter at a suitable speed. Each recorder has its own floppy disk, allowing up to 300 graphs, each of 500 points, to be stored. The data can be recalled and recorded graphically by the BS8000's own X-Y plotter. A line-printer output is also provided, along with a standard RS232C interface. A wide range of software options is available to further extend the capabilities of the BS8000.

Circle No. 135 on Reader Enquiry Card.



Gallenkamp electronic calculator/clock

GALLENKAMP

A pocket-size calculator/clock has recently been added to the Gallenkamp catalogue. In addition to all the basic mathematical functions, the calculator incorporates a clock, stopwatch, alarm, time memory, countdown alarm and a calendar. Results from the calculator are shown on an eight digit LCD display. The clock, using a crystal oscillator frequency is accurate to ±15s per month and has a 12-h digital display system. Readout is given in hours, minutes, seconds and a.m./p.m. The stopwatch of the calculator has a measuring capacity of 23 h, 59 min, 59.9 s and an accuracy of 0.1s. The calendar has been programmed up to 31 December 2099. Digital readout gives the year, month, day and date. The calculator/clock is supplied with a protective case, instructions and batteries.

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LEITZ

The capabilities of the Leitz TAS television-based image analysis system have been expanded by the introduction of powerful new modules. A 100 gray level module scans an image or individual structures under software control for density values over a selected geometry. A module with a hardware arithmetical logic unit and binary image planes, each storing a complete threshold image, combines images at high speed. It can add and subtract images and set an image as a mask. comprehensive software operating system in a built-in microcomputer. All machine funtions and image operations can be programmed in a BASIC-like language on a video terminal. The program can be stored for repeated use.

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COMTAL

The Vision One/20 is a sophisticated digital image exploitation system from Comtal of Pasadena, California. This system features real-time interactive modular components, with multi-purpose programmable processors and large data bases. The capabilities of the system can be further expanded by interfacing the Vision One/20 with the Comtal Model 8000-S solid-state refresh memory.

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G79 CATALOG YOURS FREE

Here's what's new in the Gilmont G79 Catalog. . . Expanded ranges for all Flowmeters including the Shielded versions; plus new money-saving kits to cover all the ranges. There is major expansion in the Flowmeter Micrometer Valve Assemblies, and good savings on kits there too.

Included are manometric and vacuum gages, a micrometer pipetter, micrometer syringes and syringe pipets, even a viscosimeter. And there's the new Mod. V water still with a Vycor heater. Don't miss the details on the shielded coaxial design Bypass Flowmeter. . .it's a winner. Send for your copy today.



ROGER GILMONT INSTRUMENTS, INC.

159 GREAT NECK ROAD,
GREAT NECK, N.Y. 11021
(516) 487-0120

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DISTRIBUTED COMPUTER SYSTEMS

The DCS/80 microcomputer is a low cost, industrial quality, Intel Multibus compatible development/control system. The entire system, including dual 8″ floppy disks, 5″ slot (optional 9-slot) terminated backplane and heavy duty power supply, fits in a standard 10.5″ rack space. The system includes a single board computer which is an enhanced version of the Intel iSBC 80/10. Also available is a variety of peripheral and memory options.

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ANALOGIC

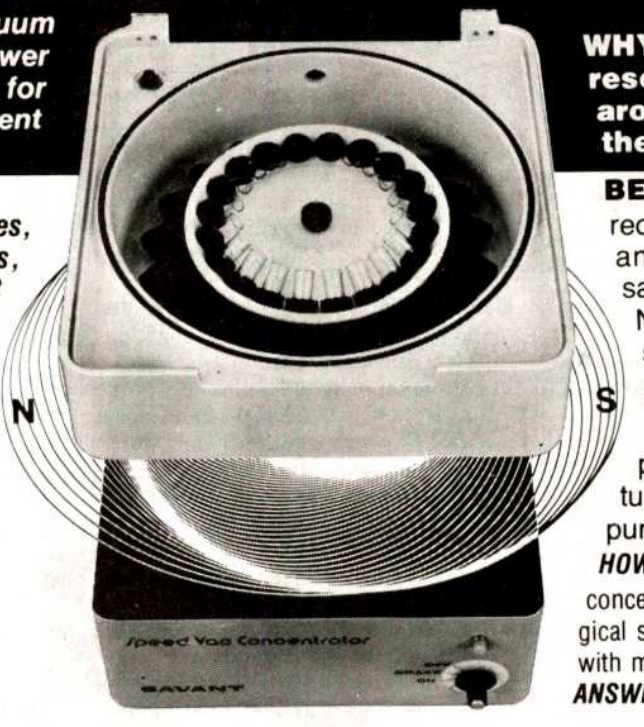
Analogic announces the development and availability of a new class of array processor, the AP400 series. The AP400 provides an exceptionally powerful array processing fully-programmable computing capability, with advanced architectural and software techniques that facilitate the implementation of real-time and/or other computational problems at low cost. The AP400 is capable of performing approximately 10 million complex floating point, high-precision computations per second. The extensive library of host FORTRAN function calls maximises programming efficiency.

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Savant Speed Vac Evaporator/Concentrator

NOW WITH MAGNETIC DRIVE –

Isolates the upper vacuum chamber from the lower motor drive section – for more efficient solvent evaporation.



WHY — are so many research laboratories around the world using the Savant Speed Vac?

Acid Hydrolysates, Eluates, Extracts, Dialysed Solutions, Preparing Samples for GLC or HPLC in Biomedical Research, Clinical Chemistry, Radioimmunoassays, Mass Spectromotry.

Use with standard culture tubes, tapered tubes, mini vials, silylation vials.

BECAUSE —100% sample recovery . . . Evaporates and concentrates at the same time...No splash... No smearing ... 20 to 100 samples at one time.

SIMPLE OPERATION — Load 20 to 100 samples in the rotor, close lid, turn to "on" and turn vacuum pump "on".

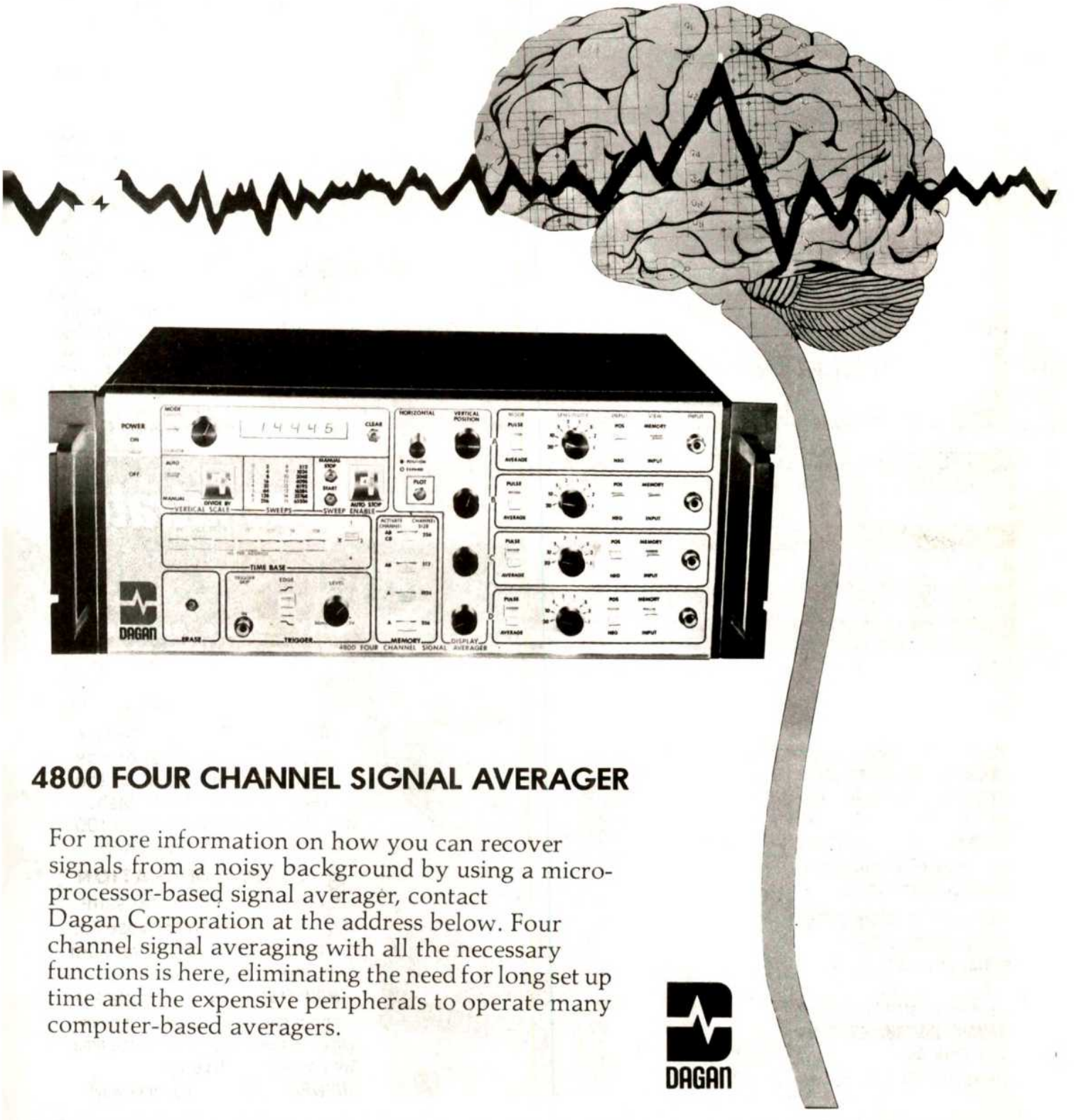
HOW ELSE can you evaporate and concentrate small volumes of biological samples in quantity at one time with maximum efficiency?

ANSWER — There is no other way!

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DAGAN for ELECTROPHYSIOLOGY



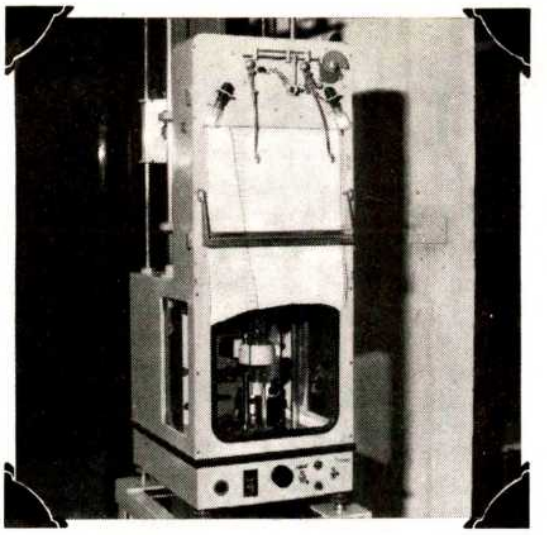
4800 FOUR CHANNEL SIGNAL AVERAGER

For more information on how you can recover signals from a noisy background by using a microprocessor-based signal averager, contact Dagan Corporation at the address below. Four channel signal averaging with all the necessary functions is here, eliminating the need for long set up time and the expensive peripherals to operate many computer-based averagers.

DAGAN

DAGAN CORPORATION 2855 PARK AVENUE, MINNEAPOLIS, MINNESOTA 55407 PHONE 612-827-5959

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5 years ago we made Britain's first recording thermobalance.

It still works.

Now, here's the world's first simultaneous thermal analyser based on an electronic microbalance.

Reliability is something Stanton Redcroft build into every instrument they make. Like the 25 year old thermobalance, or the new STA 780. It's the world's first simultaneous TG/DTG/DTA with a microprocessor-based balance control, with LED display for ease of use and visual observation, and a range of interfaces for easy linkage to computers, calculators or timers.

The new STA 780 has a water-cooled furnace for quicker turn-around time and decreased drift, with a unique micro-environment within the furnace which ensures exact location of the DTA head.

The STA 780 also features a solid state low noise D.C. amplifier – giving very reproducible DTA results – and impressive versatility afforded by a 5g total capacity.

So if you're looking for a TG/DTG/DTA system that can record all three parameters at the same time and overcome some of the problems of analysing non-homogeneous samples, look at the Stanton Redcroft STA 780.

It'll still be working in the year 2003.

Copper Mill Lane London SW17 0BN.
Telephone 01-946 7731
Telex 88 11417 STRED G

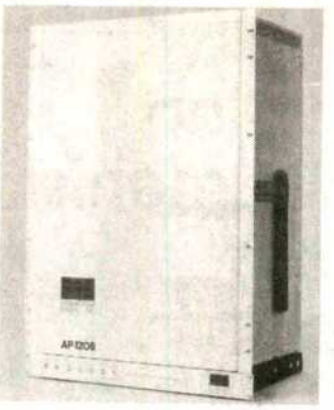


Stanton Redcroft

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FLOATING POINT SYSTEMS

The AP-120B array processor from Floating Point Systems Inc. gives economical minicomputer systems the computational power of large scientific computers. For example, an AP-120B has been used in a PDR-11/34 system to reconstruct and analyse complex digital images. Without the AP-120B, the task would take more than 2 hours. With the AP-120B, it takes less than 30 seconds — a 240 × improvement. FPS now offer a new library of scientific subroutines for use with the array processor in image processing applications. The image processing library is composed of 11 two-dimensional filtering, convolution and fast Fourier transform (FFT) routines. The routines provide array processor users with the computational tools to filter and enhance monochrome colour, and multi-spectral scanner images. As with all FPS libraries, these routines are written in highly efficient AP assembly language, but callable from host FORTRAN. Possible

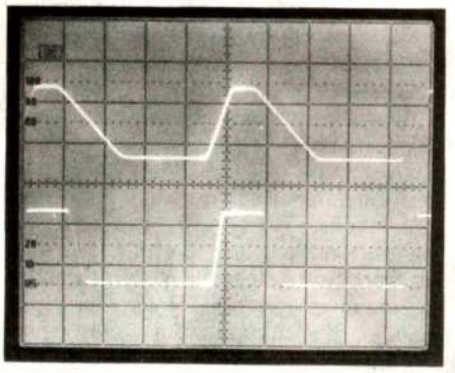


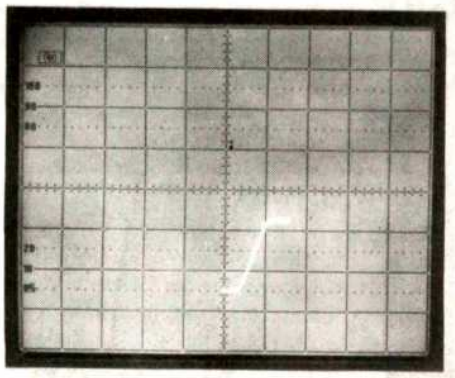
applications of the image processing library are data processing for X-ray tomography, LANDSAT and EROS satellites, high-resolution video micrography systems, and other multi-dimensional sources.

This new library is in addition to FPS's extensive application support: scientific math library, signal processing library, and advanced math library for simulation.
Circle No. 141 on Reader Enquiry Card.

HEWLETT-PACKARD

A new way of making oscilloscope measurements is available in the Hewlett-Packard Model 1741A Option 002 variable persistence storage oscilloscope. The method is based on an extension of the Lissajous figure, and presents measurements graphically as a function of two variables. It operates via a variable delay line in the horizontal axis.
Circle No. 142 on Reader Enquiry Card.





Graphic presentation of triggered X-Y measurements on the 1741A Option 002. The area of interest in the main intensified mode is selected on switching to triggered delay sweep



The HP-41C calculator forms the basis of a sophisticated computer system

The Hewlett-Packard range of advanced programmble pocket calculators includes the HP-33C, a continuous memory version of the popular HP-33E. More sophisticated is the alphanumeric HP-41C handheld calculator. As well as performing 130 pre-programmed scientific and mathematical functions, the HP-41C can be tailored to perform any number of other calculations through programs written by the user or through a wide range of plug-in applications software.
Circle No. 143 on Reader Enquiry Card.

Latest addition to the HP System 45 family of desktop computers is the HP System 45B. These are complete computer systems, combining processor, main memory, tape-cartridge storage, cathode-ray tube and optional high-speed printer in a single desktop unit. A large range of options is avaialble for data communications and peripheral additions, and the range of software is extensive.
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DAGAN CORPORATION

The 4032 digitised analog delay from Dagan process two analog signals so as to effectively allow recapturing signals usually lost due to the elapse of time. It is ideal for events which are anticipated and can be represented as a voltage waveform, but the exact occurence time is unknown.
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TIME ELECTRONICS

New from Time Electronics is the CJ10 low-cost integrator with timed print-out. It can be powered from the mains, or for up to 10 days from the internal rechargeable cell. An external car battery charged from a solar panel or wind generator can be used.
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By A. M. Petrosyants

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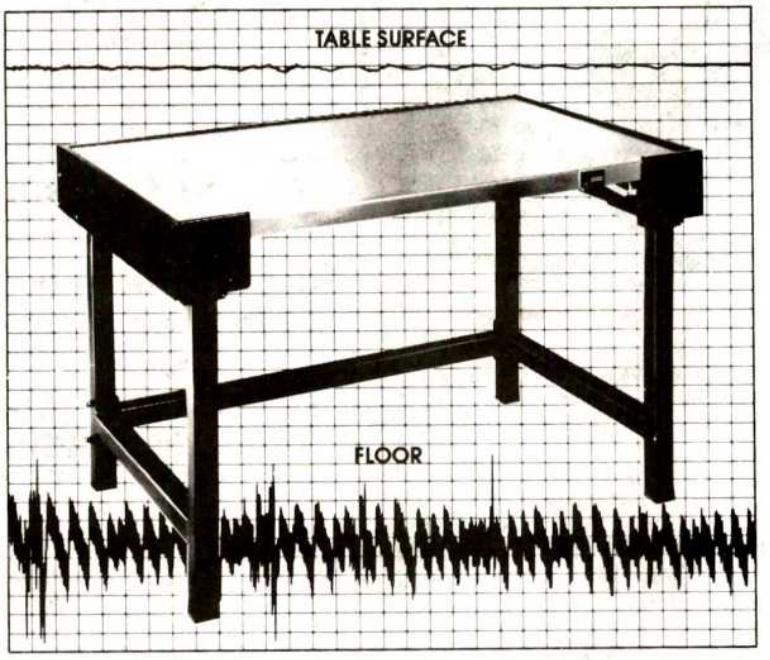
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Vibration protection for sensitive instruments! MICRO-g™ Tables and Bench Top Units.

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Standard MICRO-g Tables and Bench Top units cost approximately $1000 and will support equipment loads of over 900-lbs. All that is needed for operation is a compressed air supply or bottled air or gas.

If you're using microscopes, microtomes, interferometers, mask alignment tools or other ultra-sensitive equipment you need to know about MICRO-g systems. There are six standard configurations in a wide range of sizes. Special configurations are readily available. Write for a catalog and price list today.





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CHROMATIX

A new application note is available from Chromatix entitled 'A new data system for the polymer characterisation laboratory: application to GPC data treatment'. This 12-page note (reference LS-6) describes the use of Chromatix GPC software for branched and linear polymer characterisation. A total of 11 application notes is available from Chromatix with details on methods of molecular weight measurement and data treatment.

Circle No. 147 on Reader Enquiry Card.

BECKMAN

Providing qualitative and quantitative analysis of multi-component mixtures, Beckman's new Microlab 620 MX computing infrared spectrophotometer can provide the analytical power of a microcomputer, floppy-disk data storage centre, and totally preprogrammed and interfaced software systems at a fraction of the cost, space and training requirement of conventional instruments and data-processing systems.

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JOBIN-YVON

Two new instruments for measuring dichroism have been introduced by Jobin-Yvon of Longjumeau, France — the Dichrographes IV and V. Both are based on a microprocessor to control the performance of the instrument measuring process. Spectra can be recorded for both circular and linear dichroism with facilities including baseline correction, signal averaging. A measurements and very precise calibrations. Both instruments use the holographic gratings system developed for the Dichrographe III, covering the range 180 to 1,000 nm.

As well as the basic microprocessor the Dichrographe V contains a sophisticated minicomputer which makes it possible to perform advance curve manipulations and calculations. The BASIC programming makes it simple for the user to develop software to supplement that provided by the manufacturer. The facility for rapid scan, curve accumulation and signal averaging allow much higher sensitivity and faster response times than previously possible in circular dichroism measurements. This may make study of reactions kinetics and use of stopped flow real possibilities. Jobin-Yvon instruments are available from EDT Research.

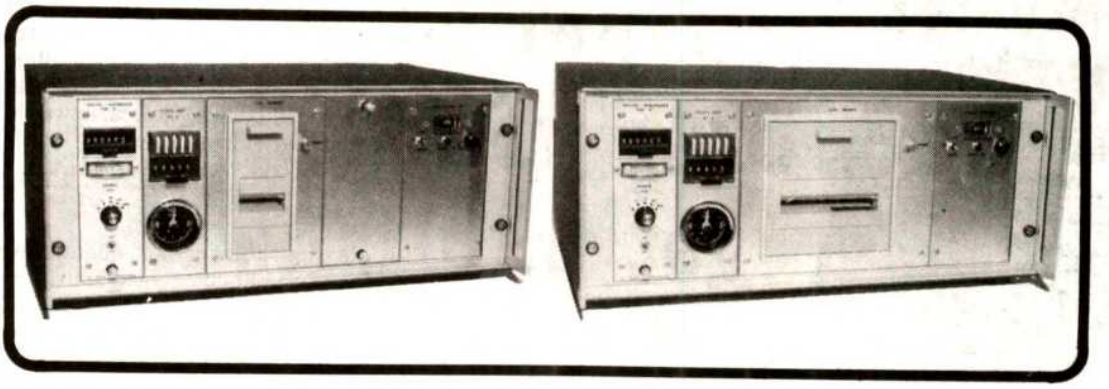
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BASCOM-TURNER

Bascom-Turner Instruments have introduced their Series 8000 recorders, which are microprocessor-based systems for data management applications. With the Series 8000 it is possible to digitise, record, and process up to eight channels of analog data. Raw or processed data are graphed in real time on strip or X-Y chart paper and stored on flexible disk.

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BATTERY POWERED INTEGRATORS TYPE CJ10/CJ20.



● LOW COST/GENERAL PURPOSE. ● BATTERY/MAINS OPERATION.

● TIMED PRINT-OUT.

The CJ10 has been designed as a low cost instrument suitable for general use where signals require integration before print-out. It is particularly suitable for remote field work since battery power enables continuous unattended operation for several months without battery charge. The internal battery will power the CJ10 for up to 10 days, while the use of an external 12 volt battery (e.g. a standard car battery) of 60Ah capacity will give continuous operation for 3 months or more at low print rates (less than 1 per 4 hours).

Applications of the CJ10 are many, however the CJ10 has been found to be particularly useful for SOLAR RADIATION measurements. With the addition of a solar radiation sensor the CJ10 will "average" the radiation strength over a timed period, a simple calculation on the printed result gives 'grams cals per sq cm per minute'.

EXAMPLE OF PRINT OUT:

6 5 4 3 2 1
6 5 4 3 2 1

CJ10 PRINT-OUT

6 5 4 3 2 1 4 5 1 23 5 9
6 5 4 3 2 1 4 5 1 23 5 9
DATA **DAY NO. TIME**

CJ20 PRINT-OUT

The CJ20 differs only in print format from the CJ10. The print out from the CJ20 has day number and time printed along side the data (see examples above) this feature is very useful if records are to be kept over a long period of time, it also makes manual prints easily identifiable on the print-out.

PROTECTIVE CASE

Provides extra protection when the instrument is being used in other than laboratory conditions. Particularly suitable for dusty or humid climates. The case is lockable to prevent unauthorised adjustments etc.

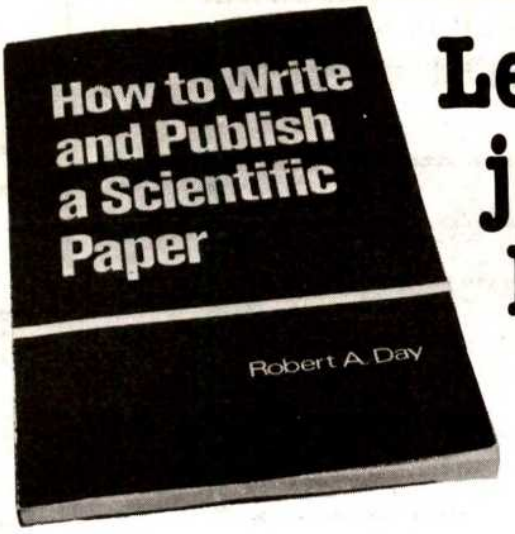
AUTOMATIC CONTROL UNIT TE9570

This unit allows external equipment such as irrigation controllers etc to be driven. The unit plugs into the CJ10 frame and provides a contact closure for 300 mS when a preset count is reached, after which the unit resets to the set value and continues cycling. Maximum preset value 99999. (The TE9570 is external with CJ20).

TIME ELECTRONICS LTD.
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TIME ELECTRONICS

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Consider this: many of the better scientific journals reject 50% or more of the papers submitted. And all too often the cause for rejection isn't bad science, but bad presentation.

In *How to Write and Publish a Scientific Paper,* Robert A. Day has written a handbook which should substantially improve the would-be author's probability of publication. Day is certainly well qualified to address the subject: for the past 18 years he's been Managing Editor of nine journals published by the American Society for Microbiology.

The book itself is a demonstration of clear and concise presentation. Step by step, it covers every aspect of the writer's task—from preparing a title (more important than most people think) to dealing with editors and printers. The chapters on the use and abuse of tables and illustrations and on interpreting letters of rejection are particularly instructive.

Throughout, the point of view is that of a working editor. The style is lively, the treatment often amusing; but the content is thoroughly serious. Much of the information offered here is not readily available elsewhere.

For certain, this is a book you will use—whether you read it from cover to cover or reach for it in times of desperate need. In the words of one reviewer,* "[it] should be in the hands of young, middle-aged, and old scientists. It should be on the desk of everyone who aspires to write a scientific paper."

Available direct from the publisher: $15.00, clothbound; $8.95, paperback. 160 pages, illustrated, with six appendixes and an index.

*Albert Balows, Editor-in-Chief, *Journal of Clinical Microbiology.*

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Recent scientific and technical books

Applied biology

WALLER, T. Gary, and MACKINNON, G.E. Reading Research: Advances in Theory and Practice. Pp.x + 262. ISBN-0-12-572301-6. (New York and London: Academic Press, 1979.) $18.50.

WITTIG, Michele Andrisin, and PETERSEN, Anne C. (ed.). Sex-Related Differences in Cognitive Functioning: Developmental Issues. (Academic Press Series in Cognition and Perception.) Pp.xvi + 378.ISBN-0-12-761150-9. (New York and London: Academic Press, 1979.). $24.50.

WECKELGREN, Wayne A. Cognitive Psychology. Pp.xi + 436. ISBN-0-13-139543-2. (Englewood Cliffs, NJ.: Prentice-Hall, Inc., 1979.)

Sociology

BIERI, Peter, HORSTMANN, Rolf-P., and KRUGER, Lorenz (edited by). Transcendental Arguments and Science: Essays in Epistemology. (Synthese Library, Vol. 133.) Pp.viii + 414. ISBN-90-277-0964-5. (Dordrecht; Boston, Mass. and London: D. Reidel Publishing Company, 1979.) Dfl.30; $14.95.

BOEHM, Klaus, and WELLINGS, Nick. The Student Book 1979-80: The Discriminating Students' Guide to UK Colleges, Polytechnics and Universities. Pp.viii + 424. ISBN-0-0333-25918-1. (London and Basingstoke: The Macmillan Press, Ltd., 1979.) £2.95 net.

BORGIOLI A. and CAPPELLI G. The Living Swamp. Pp.vi + 120. ISBN-0-85613-012-5. (London: Orbis Publishing Ltd., 1979). £5.95.

BUCKLES, Mary Parker. Animals and Their World. Pp.240. ISBN-0-7137-1027-6. (Poole, Dorset: Blandford Press, Ltd., 1979.) £8.95 net.

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General

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POST-DOCTORAL RESEARCH ASSOCIATE NUCLEAR PHYSICS

A post-doctoral research associate position is open in the application of nuclear physics techniques to mass spectrometry employing a 3 MV tandetron as a molecular dissociator. Applicants should have experience in nuclear physics with electrostatic accelerators and the instrumentation of nuclear physics.

The position is for a maximum of three years and involves the supervision of the construction and the installation of the apparatus and further development of the systems for radio carbon dating, geological ion microprobe work and nuclear physics.

Send resume and two letters of reference to: Prof. D.W. Strangway, Department of Geology, University of Toronto, Toronto, Ontario, M5S 1A1. W541(A)

MATHEMATICIANS

with active research interests in either numerical analysis (emphasis on partial differential equations or finite element methods or applied modeling (emphasis on inter-disciplinary interest in natural/biological sciences) are invited to apply for

TENURE-TRACK POSITIONS ASSISTANT PROFESSOR LEVEL

to start September, 1980. PhD with undergraduate/graduate teaching/thesis direction competence required.

Send curriculum vitae and names of 3 references by February 1, 1980, to: Professor Sherman C. Lowell, Department of Pure and Applied Mathematics, Washington State University, Pullman, Washington 99164.

An Equal Opportunity/Affirmative Action Employer. W546(A)

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF PATHOLOGY

The Department of Pathology, University of Cambridge wish to appoint a post-doctoral scientist to join an MRC team led by Dr H Waldmann. The programme is concerned with the Immunology of Bone Marrow Transplantation in man and mouse. Emphasis will be on the exploitation of monoclonal antibodies and fluorescence activated cell sorting in conjunction with conventional techniques of experimental haematology.

This will involve extensive collaboration with the Laboratory of Molecular Biology. Candidates aged under 30 should be appropriately qualified. Salary up to £6,066 p.a. The appointment is for 3 years in the first instance.

Please send Curriculum Vitae together with names of two referees to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. 3795(A)

UNIVERSITY OF HAWAII — MANOA

POPULATION GENETICS LABORATORY

RESEARCHER GENETIC EPIDEMIOLOGY

Full-time investigator in one of several areas relevant to inherited causes of disease in populations. Doctoral degree necessary. Salary and rank commensurate with experience.

Send curriculum vitae to: Dr Newton E Morton, Director, Population Genetics Laboratory, 1980 East West Road, Honolulu, HI 96822. W543(A)

UNIVERSITY OF CALIFORNIA, Berkeley

COLLEGE OF NATURAL RESOURCES

Faculty and Agricultural Experiment Station Position in the Divsion of Biological Control and Department of Entomological Sciences.

A position as

ASSISTANT PROFESSOR OF ENTOMOLOGY and ASSISTANT ENTOMOLOGIST

in the Experimental Station will be available 18 January, 1980. A PhD in a biological sciences with specialization in invertebrate ecology is required.

Qualifications must include training in insect population ecology applicable to biological control, insect ecology, and population ecology; skills in statistical and quantitative evaluation and computer methodology would be an asset. Research responsibilities (80%) in the Division of Biological Control will include field and experimental ecology, working with live insects, with emphasis on biological control, assessment of efficiency of entomophagous arthropods in relation to both physical and biotic environmental factors.

The assignment includes conceptualization, organization and conduct of classical biological control, assessment of pest potential for economic damage and of naturally-occurring biological control, and experimentation on predator-prey/host-parasite relationships. Teaching (20%) is in the Department of Entomological Sciences in insect ecology, especially population ecology, and biological control.

Applicants should send full curriculum vitae, relevant publications and manuscripts, official academic transcripts, the names of three persons as reference, and a statement on research interests by 18 January 1980 to:

L E Caltagirone, Chairman, Division of Biological Control, University of California, 1050 San Pablo Avenue, Albany, California

The University of California is an affirmative action employer. W545(A)

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE

ASSOCIATE DIRECTOR Science and Technology Policy Programme

Applications are invited for the post of Associate Director for the Science and Technology Policy Programme of the International Development Research Centre (Canada). The Associate Director who will be based in Ottawa will report to the Director of the Social Sciences Division and will be responsible for IDRC's programme of support for science and technology policy research in the developing world.

The successful applicant will undertake the following duties: 1) Develop and monitor research projects in the Science and Technology Policy Programme in Asia, Africa and Latin America. 2) Evaluate research proposals from developing countries in science and technology policy. 3) Supervise the preparation of project proposals and documentation undertaken by Programme Officers. 4) Prepare annual Programme budget estimated at $1,500,000. 5) Advise the Director on future Programme directions and priorities.

Qualifications required: 1) PhD with appropriate specialization. 2) Familiarity with a broad range of science and technology policy research issues in developing countries. 3) Previous experience with international agencies desirable. 4) Relevant overseas experience. 5) Working capacity in either Spanish or French desirable. Salary commensurate with qualifications and experience.

Applications with the names of 3 referees should be submitted to the Director, Social Sciences Division, IDRC, PO Box 8500, Ottawa by December 31, 1979. 3793(A)

IMMUNOLOGIST

Boehringer Mannheim is a leading research-orientated pharmaceutical company in Western Germany. A research position is immediately available in the Cell Culture Division of our Biochemical Research Centre, Tutzing near Munich. The candidate will be expected to build up a research group in the field of hybridoma technology.

Previous experience in cellular immunology, cell culture techniques, hybridoma production, cell cloning and anti-body analysis is essential.

Applicants without a PhD but with appropriate experience will also be considered.

Boehringer Mannheim offers an excellent salary and an attractive benefits package.

Interested applicants should send curriculum vitae, list of publications and salary requirements to

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— Personalabteilung —
Bahnhofstraße 9-15, D-8132 Tutzing

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W559(A)

RESEARCH OPPORTUNITIES

Birds in an agricultural and airfield environment



These posts are in the Land Pests and Bird Research Department near Guildford which undertakes ecological, biological and pathological studies with a view to controlling harmful birds and mammals. There are excellent library and experimental facilities.

ETHOLOGIST

. . . to lead a small team studying the foraging strategies and food preferences of birds, particularly bullfinches, under natural conditions with a view to developing repellants for use in crop protection. This will involve planning and carrying out aviary and field experiments and publishing results.

Candidates, normally aged under 30, should have a good honours degree or equivalent in an appropriate discipline. At least 2 years' postgraduate experience in the study of animal behaviour and a sound knowledge of statistical methods is essential. An interest in optimal foraging theories and experience of working with birds is highly desirable. Appointment as Higher Scientific Officer. *Ref: SB/41/AF.*

ORNITHOLOGIST/ ECOLOGIST

. . . to initiate a study of the relationship between the roosting and feeding habits of starlings in agricultural habitats. The work will also involve analysing the factors that may influence the selection of feeding grounds, assessing the profitability of producing cheap alternative food for starlings in an attempt to reduce damage to economically important crops, investigating breeding control methods and preparing results for publication.

Candidates, normally aged under 27, should have a good honours degree or equivalent in an appropriate discipline. Appointment as Scientific Officer. *Ref: SB/42/AF.*

ORNITHOLOGIST

. . . to join a small team studying the behaviour of birds in airfield environments and advising on the potential danger to aircraft. The work will involve collecting and analysing data on populations and movements of lapwings whose affinity for airfields by both day and night is well established but little understood. In addition, the successful candidate will improve methods to alleviate bird hazards, advise civil and military aviation authorities on ornithological problems and instruct airfield personnel on bird control techniques.

Candidates, normally aged under 27, should have an honours degree or equivalent in an appropriate discipline. Appointment as Scientific Officer. *Ref: SB/43/AF.*

Salary (from 1.1.80) for Higher Scientific Officer ranges between £5,095 and £6,735 and for Scientific Officer between £3,590 and £5,485. Promotion prospects. Non-contributory pension scheme.

Candidates who applied for similar posts earlier this year are invited to apply again if they still wish to be considered. Men and women may apply.

For further details and an application form (to be returned by 14 December 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote appropriate reference.*

3800(A)

Agricultural Science Service, Worplesdon Laboratory, Surrey.

Science Group CIVIL SERVICE

RESEARCH TECHNICIAN Specializing n endocrine research with specialized xperience in catecholamines. Duties nclude conduction radioenzymatic assays for catecholamines. Experience in RIA work and Preparative Biochem. BS in Biology or Chemistry required. Salary $1250.00/month. Send resume to: Raul Artal, MD, 1240 N Mission Rd, 5K-22, Los Angeles, CA 90033. W552(A)

UNIVERSITY OF BATH
SCHOOL OF PHARMACY & PHARMACOLOGY
POST-DOCTORAL RESEARCH OFFICER IN MEDICINAL CHEMISTRY

Applications are invited for the above post rom graduates in Pharmacy, Chemistry or Biochemistry, with postgraduate ualifications and experience. The uccessful candidate will work with rofessor R T Parfitt on Novel Centrally acting Tropanes. A good knowledge of rganic synthesis and modern spectro-copic techniques is essential and some ackground in either medicinal chemistry r pharmacology is desirable.

The post is tenable for two years with a arting salary of up to £5,199 per annum nder review).

Application forms and further articulars from the Personnel Officer, niversity of Bath, Bath, BA2 7AY, uoting reference number 79/217R. losing date will be 7th December, 1979.

3779(A)

SENIOR TOXICOLOGIST

Owing to extending needs and facilities in toxicology (new laboratories will open soon), we are looking for a Senior Toxicologist.

Applicants (normally aged under 40) must have a doctoral degree (MD, or VMD, or PhD). Qualification as pathologist and knowledge and/or experience in carcinogenicity and mutagenicity fields are desirable. Experience of contacts with official regulatory organizations would be an important consideration.

Reporting directly to the Toxicology Department Head, the successful applicant will take over the supervision of the toxicological evaluations conducted in outside facilities. He is also expected to take an active role in official contacts and representation in scientific or professional organizations. Fields to be covered: pharmaceuticals, veterinary products, animal feed.

Fluent English is mandatory. French is not required at the time of application but the engaged candidate will have to learn it.

Position to be filled near Paris. Salary depending upon qualification and experience.

Please send application including curriculum vitae and credentials to *Nature*, quoting box number W539A, c/o Nature, 3, Dyers Buildings, London EC1N 2NR.

W539(A)

LINCOLN COLLEGE
(University College of Agriculture)
New Zealand

LECTURER IN CROP PHYSIOLOGY

Applications are invited for the position of Lecturer in the Department of Plant Science. Applicants should have a postgraduate qualification and research experience in crop physiology, preferably combining a sound knowledge of physiological principles with personal experience of crop pasture production. Teaching at undergraduate and post-graduate levels will necessitate competence in such topics as photosynthesis, water relations, competition, etc.

Commencing salary according to qualifications and experience. Present salary scale for lecturing staff is: NZ$13,722 to NZ$16,861.

Travel and removal expenses are reimbursed up to specified limits. New Zealand Government Superannuation available.

Conditions of Appointment are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University. Applications close on 15 January 1980. Please quote Vacancy No. 78/50.

3774(A)

POSITIONS OPEN
Medical University of South Carolina
DEPARTMENT OF BIOCHEMISTRY

Three positions are available from 1 January, 1980.

1. ASSISTANT PROFESSOR

with strong research background in biochemistry of lymphocyte recognition and function. The individual should be an effective lecturer in this area and be capable of generating research support. Several years of postdoctoral experience are required.

2. ASSISTANT PROFESSOR

with proven research and teaching ability in the area of demonstration and characterization of cell surface receptors. Several years of postdoctoral experience are required and the individual should be capable of developing a fundable research program in this area.

3. ASSOCIATE PROFESSOR

with established research program in molecular immunology. Research interests should center around the evolution of antibodies and cell receptors. The individual should be an effective teacher in these areas.

Closing date for applications is 30 November, 1979. Send curriculum vitae to Dr J J Marchalonis, C/O Office of the Dean, College of Medicine, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29403. W553(A)

Water Quality Department

CHIEF ASSISTANT CHEMIST

Location: **Initially at Lichfield, permanent location at new Central Laboratory, Walsall, West Midlands**

Salary: **NJC Water Services Staff Grade 10 Range: £9,096 — £9,771 Plus Regular Car User Allowance**

Applications are invited for the above post from professionally qualified chemists, aged 30 to 40, holding a good honours degree in an appropriate scientific subject. Technical experience may relate directly to the water industry or to a broadly similar area and applicants should have a good command of management skills.

The person appointed will assist the Chief Chemist and Deputy Chief Chemist in the full range of departmental responsibilities which include the control of water treatment and the provision of laboratory analytical facilities. The laboratory's analytical duties include comprehensive chemical, biological and bacteriological analyses necessary for the provision of a wholesome water supply. Advanced instrumentation is available for a wide range of these analyses and is to be extended when the laboratory is transferred to new accommodation. The development of the laboratory's facilities is at a particularly challenging stage at the present time, in relation to data handling systems.

The Company offers approved relocation expenses where appropriate and first class superannuation provision. Opportunities for further advancement exist for a candidate who can demonstrate exceptional technical and managerial ability and a progressive and enthusiastic approach to his work.

Applications, including comprehensive details of qualifications, experience, and present responsibilities and salary, together with the names of two referees, should be marked "Confidential" and sent by 11th December to the General Manager, The South Staffordshire Waterworks Company, 50 Sheepcote Street, Birmingham B16 8AR.

3775(A)

UNIVERSITY OF LEIDEN
The Netherlands Faculty of Science

The Subfaculty of Chemistry of the University of Leiden, The Netherlands, invites applications for the tenured position of

ASSOCIATE PROFESSOR OF BIOPHYSICAL CHEMISTRY (M/F)
vacancy number 9-480/2407

The appointee is expected to focus his/her research on the structure and dynamics of biological macromolecules and molecular assemblies with the aid of physical methods. It is also expected that the appointee will stimulate and coordinate cooperative research efforts in this field between the department of biochemistry and other disciplines.

Responsibilities will include teaching.

Applications with curriculum vitae and including full details of qualifications and experience should be addressed to the University of Leiden, Personnel department, Stationsweg 46, Leiden, The Netherlands.

RU leiden

Information:
Dr A van der Gen,
Gorleaus Laboratoria,
University of Leiden,
Netherlands.

W538(A)

SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING
SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

A Scientific Officer is required to join the Cereals Department at the Scottish Plant Breeding Station.

The appointee will be required to join a group working with spring barley and spring oats with the object of producing crop varieties adapted to local conditions. Training in genetics and/or pathology along with an interest in the practical aspects of plant breeding would be an advantage.

Qualifications: A pass degree or HNC with relevant experience or an honours degree in a biological science. At least five years relevant post-graduate experience is normally required for appointment as HSO.

Salary: Depending on qualifications and experience:
Scientific Officer: In the range £3,289 to £5,085 per annum.
Higher Scientific Officer: In the range £4,726 to £6,211 per annum.
The post is superannuable.

Application forms and further information may be obtained from the Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF, to whom completed application forms should be returned not later than 3rd January 1980.

3735(A)

IMPERIAL COLLEGE
Silwood Park, Ascot, Berks. SL5 7PY

INSECT BEHAVIOUR

A POST-DOCTORAL RESEARCH ASSISTANT

is required at the Department of Zoology and Applied Entomology to work on tsetse fly behaviour. Candidates should have a PhD or equivalent, preferably with research experience, in insect behaviour or sensory physiology. The project is part of a long-term study of the visual responses and circadian rhythms of tsetse flies.

Salary within the range £4,333 to £5,488 per annum (salary under review) plus USS benefits and 5 weeks holiday. The appointment is for one year in the first instance, renewable for a further year. Extension beyond that will depend on the success of the project and the outcome of future grant applications. Starting date 1 February 1980.

Apply in writing to Dr J Brady (above address), with curriculum vitae and the names and addresses (plus phone numbers) of two referees, before December 21st.

3804(A)

UNIVERSITE DE MONTREAL
FACULTÉ DES ARTS ET DES SCIENCES
Département de sciences biologiques

PROFESSEUR A PLEIN TEMPS

Fonctions: Enseignement de la biométr de la dynamique des populations et modélisation. Encadrement des étudian de 2e et 3e cycles. Participation aux trava de recherche et aux activités d département.

Exigences: PhD et expérience recherche en dynamique des populations en modélisation. Très bonne connaissan de la langue française.

Traitement: Selon les normes de convention collective en vigueur. Da d'entrée en fonction: 1er juin 1980 ou plus tôt possible après cette date. Date clôture des candidatures: 1er mars 1980.

Les candidats sont priés de faire parve leur curriculum vitae à: M. Jean-G Pilon, Directeur, Département de scienc biologiques, Faculté des arts et d sciences, Université de Montréal, Ca postale 6128, Succurssale A, Montr (Québec), H3C 3J7. Tel.: (514) 343-687

W556(A)

We have the following vacancies in our Biochemistry Section:

Experimental Officer — Biochemist

A graduate biochemist to play a part in the discovery of new insecticides. An understanding of neurobiology would be desirable, but practical experience in insect biochemistry is not essential. However, candidates must have considerable experience of modern biochemical techniques.

The successful applicant will join a small team composed of one PhD and one graduate, responsible for initiating research in areas of insect biochemistry that could lead to a programme of chemical synthesis or produce *in vitro* methods for detecting active chemicals. Complementary work will be concerned with evaluating the mode of action of promising new insecticides and gaining an early understanding of the factors that may contribute to the development of resistance.

Experimental Officer — Physical Chemist/ Electrochemist

A Physical Chemist to work on the electrochemistry and reaction kinetics of exploratory organic compounds being synthesised as potential pesticides. A knowledge of chemistry at least up to HNC level is required, and experience of polarography or related techniques is desirable but not essential. The successful candidate will work closely with organic and bio-chemists, and will be in a small team headed by a PhD physical chemist. There will be scope for initative in developing techniques and helping to design structures with the desired electrochemical properties.

Jealott's Hill is the chemical and biological research centre for the crop protection interests of ICI. It is situated in open country 30 miles west of London, between Bracknell and Maidenhead. We offer competitive salaries and fringe benefits including membership of the ICI Profit Sharing and Pension Fund. Coaches run between Jealott's Hill, Bracknell and Maidenhead.

If you are interested in this vacancy please write or telephone for an application to:

Mr S R Stephenson, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berks. Ref no 79/Biochem

ICI Plant Protection Division

3794(A)

FACULTY POSITION

Organic Chemistry

Applications are invited for a tenured senior appointment which the Department of Chemistry at Stanford University expects to be able to make in the near future. Applicants must have outstanding research credentials in organic chemistry and a strong commitment to teaching undergraduates as well as graduates.

In applying, send curriculum vitae and bibliography to: Professor K.O. Hodgson, Department of Chemistry, Stanford University, Stanford, California 94305. Stanford is committed to Equal Opportunity through Affirmative Action in employment and we are especially eager to identify women and minority persons with appropriate qualifications.

Applications should be received by 15 December, 1979. W516(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT 1A

Ref: B/R/10

Applications are invited for the position of Post-Doctoral Assistant (Grade 1A) funded by a 3 year project grant from the MRC awarded to Dr A J Kingsman. The research concerns the characterisation of dispersed repetitive DNA sequences in the genome of *Saccharomyces Cerevisiae* by both genetic and recombinant DNA techniques. The appointment should start as soon as possible after 1st January, 1980.

Salary on the scale £4333 — £6066.

Applications, including the names of 2 referees should be sent to: The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. 3808(A)

ROCHE

Senior Pharmacologist

Roche Research Institute of Marine Pharmacology (R.R.I.M.P.)

invites applications for the position of

Section Leader Pharmacology

Roche has established a Research Institute of Marine Pharmacology in Sydney to carry out basic research into the biology, chemistry, microbiology, chemotherapy and pharmacology of a wide range of natural products obtained from marine organisms. There is a close interaction between the scientific sections. The Institute collaborates with Australasian universities and its operation is integrated with that of other Roche research centres in Switzerland, the U.K., Japan and the U.S.A.

The Section Leader will direct the Pharmacology programme which evaluates activities of material isolated from marine organisms.

Responsibilities will include development and coordination of research projects in the Pharmacology section, appropriate to the function of the Research Institute, interaction with clinical pharmacologists in specific projects, and interaction with such authorities as are necessary for the development of a drug.

The appointee will advise the Director on development of the Pharmacology section and will be a member of the RRIMP Research Management Committee.

Applicants should have demonstrated the ability to organise and supervise a scientific team involved in both routine and research application.

Salary will be negotiated on the basis that the level of appointment is regarded as comparable in expected expertise and responsibility to that of a Reader or Associate Professor in a University or Senior Principal Research Scientist in a Commonwealth Research organisation.

Generous superannuation benefits are available.

Applications marked confidential, and including curriculum vitae and the names of three referees, should be forwarded to:

The Director,
Roche Research Institute of Marine Pharmacology,
P.O. Box 255,
DEE WHY. N.S.W. Australia 2099.

Closing date is: 14th January, 1980.

3786(A)

Cytogeneticist

Chief Medical Officers Department

Up to £7,500-Windscale

British Nuclear Fuels Limited rely on specialists—professionals who can fill specific roles vital to our continuing success, consolidating our position as a conscientious world leader in a challenging industry.

Right now we require a graduate with at least 2 years experience in human genetics preferably including tissue culture and banding techniques to join our expanding laboratory.

The work is primarily concerned with the evaluation of chromosome aberrations in relation to radiation dosimetry together with a programme of research in this field. In addition a close liaison is maintained with the hospital services in the area for whom some diagnostic work is undertaken.

We have a superb location—right on the West Cumbrian Coast and on the fringe of the lake District National Park. This picturesque area provides a eutopian combination of lakeland, hills, forestry and coastline—offering a relaxed and charming way of life with excellent opportunities for outdoor pursuits.

We offer an excellent salary, up to £7,500, plus a productivity bonus, and benefits include generous resettlement allowances in appropriate cases. Housing, both rural and urban, is readily available at realistic prices and company accommodation may be obtainable.

To apply, write now with details to Dr. G.B. Schofield, Chief Medical Officer, British Nuclear Fuels Limited, FREEPOST, Windscale and Calder Works, Sellafield, Seascale, Cumbria.

BNFL

For an infinitely brighter future

3787(A)

UNIVERSITY OF MANCHESTER

LECTURESHIP IN PHYSICS

Applications are invited for the above post. The Lecturer will be expected to take part in teaching in the Physics Department and to carry out research on Experimental Elementary Particle Physics, in either the JADE Collaboration on the PETRA e^+e^- storage ring at Hamburg or the Photon Collaboration on the OMEGA detector at the SPS at CERN, Geneva. Further information can be obtained from Professor P G Murphy. Salary within the range £4,333 to £8,992. Superannuation. Initial salary will depend on age and experience but is unlikely to be above £5,199.

Applicants with appropriate experience in experimental particle physics should apply to the Registrar, The University, Manchester M13 9PL, from whom further particulars and application forms are obtainable. Completed forms should be returned by December 15th. Quote ref: 234/79/N. 3730(A)

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research

DIVISION OF VIROLOGY

A vacancy exists for a researcher to join a small group investigating the basic mechanisms of virus infection using the adenovirus model system. Ideally, he/she should have had some years' postdoctoral experience in a similar or related field. The appointment would be a temporary one for three years but, given sufficient experience and evidence of research abilities, a five year limited-term appointment (which could progress to a permanent post) would be considered. The salary would depend on age and experience in the range £5,199 to £6,932 pa plus £740 pa London Allowance. Superannuation provision.

Applications, enclosing curriculum vitae and the names of two referees, should be sent to the Director, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, before 14th December, 1979. 3776(A)

UNIVERSITY OF WESTERN AUSTRALIA

Perth

SENIOR TUTOR IN PHYSICS

Applications are invited for two positions in the Department of Physics. These will be temporary appointments, one for an initial period of two years with the possibility of extension, and the other to 31st December, 1980. Applicants should hold a higher degree in physics and have had appropriate teaching experience at tertiary level. They should be prepared to take a full part in the Department's teaching programme, but the appointees will also be expected to participate in one of the current research projects, which include Elementary Particles, General Relativity, Detection of Gravitational Radiation, Solid State Physics, Atmospheric Physics, Mass Spectrometry, Molecular Collisions and Photonuclear Reactions.

Current salary range: $A14,041 to $A16,085 per annum. An appointee for two years will be entitled to fares to Perth for self and fully dependent family and removal allowance, and to similar return benefits to place of engagement on completion of two or more years' service. An appointee for one year will be entitled to an allowance of up to $A2,066 towards appointment expenses.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Acting Staffing Officer, University of Western Australia, Nedlands, 6009, Western Australia, by 8th December, 1979. Candidates should request three referees to write immediately to the Acting Staffing Officer. 3773(A)

KINGSTON POLYTECHNIC

SCHOOL OF GEOLOGY

SLIDE MAKER

To be responsible for all thin section preparation for the School, together with such ancillary techniques as preparation of polished ore specimens, specimen slabbing, and preparation and polishing of grain slides. Experience essential; Advanced technician certficiate an advantage.

T3/4 grade £4470-£5457 inclusive. Application forms from Assistant Registrar (Personnel), Kingston Polytechnic Penrhyn Road, Kingston-upon-Thames KT1 2EE. 01-549 1366. 3788(A)

FACULTY POSITION IN BIOLOGICAL SCIENCES

at the Hopkins Marine Station of Stanford University

Applications are invited for an appointment in the Department of Biological Sciences of Stanford University, to be resident at the Hopkins Marine Station in Pacific Grove, California. The position is without restriction as to rank although some preference will be given to younger candidates.

The position involves both teaching and research. The areas in which the Station wishes to expand its faculty include Cellular, Neuro- and Developmental Biology. All applications must include a curriculum vitae, a statement of research interests and a list of three people from whom letters of reference will be sought.

Applications should reach C S Pittendrigh, Hopkins Marine Station, Pacific Grove, CA 93950 by January 15, 1980. Stanford University is an equal opportunity employer. W505(A)

UNIVERSITÄT FREIBURG

DEPARTMENT OF BIOLOGY

TWO POST-DOCTORAL POSITIONS

(BAT IIa: approx. 42,000 DM/year) will be available for at least two years at the beginning of 1980 for research on light-dependent development of barley and on cloning of cereal DNA.

Applications with curriculum vitae and names of two referees should be sent to Dr K Apel or Dr G Feix, Institut für Biologie, Schänzlestraße 1, 7800 Freiburg, W. Germany. W560(A)

THE UNIVERSITY OF BRITISH COLUMBIA

CLINICAL ENGINEERING PROGRAM

Applications are invited for the position of

DIRECTOR OF THE CLINICAL ENGINEERING PROGRAM

The Director's responsibility will be the operation and development of a Master-level program approved to start in September, 1980. The Director must hold an advanced engineering degree (PhD preferred), have several years clinical experience and be eligible for registration as a Professional Engineer in British Columbia. The appointment will be at the Associate Professor level and applications should be sent, by January 15, 1980, to: Professor Axel Meisen, Chairman, Clinical Engineering Search Committee, Faculty of Applied Science, University of British Columbia, Vancouver, BC V6T 1W5, Canada. (Late applications may be considered. W549(A)

UNIVERSITY OF GLASGOW

DEPARTMENT OF ELECTRONICS AND ELECTRICAL ENGINEERING

RESEARCH ASSISTANT

(Readvertisement)

Applications are invited from well qualified persons to take part in a Ministry of Defence sponsored investigation into the growth of indium phosphide by molecular beam epitaxy.

Applicants should have at least three years postgraduate research experience in one or more of the following topics: thin film deposition; assessment of III-V semiconductor materials; surface physics and related techniques. It may be possible for a suitably qualified person to register for a higher degree.

Starting salary up to £5,488 on the grade 1A scale for research staff.

The appointment is tenable for two years.

Applications (two copies) should be sent to Professor John Lamb, Department of Electronics and Electrical Engineering, The University, Glasgow G12 8QQ, giving the names and addresses of at least TWO persons to whom reference can be made.

Closing date: 31st December 1979.

In reply please quote Ref.No.4551M. 3806(A)

Director, Institute of Biological Sciences

SYNTEX, a unique Multinational Pharmaceutical Corporation, is located in the hills of Palo Alto, California, adjacent to Stanford University.

Applications are now being accepted for the position of Director of our Institute of Biological Science from individuals with broad research experience in biological science and drug development. This position reports to the Sr. Vice President of Syntex Research.

The individual selected will help ensure the highest quality of research in the Institute and will contribute directly to the long range success of the Company.

Responsibilities include directing the efforts of a number of groups of Biological and Biochemical Scientists working in the areas of inflammation, immunology, thrombosis and atherosclerosis, dermatology and reproductive physiology. Projects involve drug testing, study of mechanism of action of therapeutic agents and basic biology of diseases.

Your broad overview of Biology and Medicine and your interdisciplinary approach to problem solving are key qualifications for this position. You will function in close collaboration with other Syntex Institutes (e.g., Clinical Medicine and Pharmacology) and will exercise your strong managerial and motivational skills. Experience in the Pharmaceutical Industry highly desirable.

This Senior-level position offers a most attractive salary and compensation package along with continued professional recognition and challenge.

Please address your resume, in confidence, to John Hudson, Director of Personnel, SYNTEX, 3401 Hillview Avenue, Palo Alto, CA 94304. We are an equal opportunity employer. W554(A)

SYNTEX

UNIVERSITY OF LIVERPOOL

CYTOGENETICS UNIT

SCIENTIFIC OFFICER

Applications are invited from Science graduates for a basic grade post on the Whitley A Scales in this Unit, which provides the Cytogenetics service for the Mersey Regional Health Authority. This post primarily involves work in pre-natal diagnosis and experience in human cytogenetic techniques/tissue culture is desirable.

Salary within range £4,035 to £4,935 pa depending on qualifications and experience.

Application forms may be obtained from The Registrar, The University, PO Box 147, Liverpool, L69 3BX. Closing date for return of forms 3rd December, 1979. Quote Ref: RV/845/N. 3777(A)

THE SIDNEY FARBER CANCER INSTITUTE and the HARVARD MEDICAL SCHOOL seek an ASSOCIATE OR FULL PROFESSOR OF PHARMACOLOGY to head a division of PHARMACOLOGY

at the Institute. The incumbent should have research interests and accomplishments related to pharmacology or biochemistry.

Candidates should send a curriculum vitae and a statement of their research interests to Dr Peter Goldman, Chairman of Search Committee, Department of Pharmacology, Harvard Medical School, 250 Longwood Avenue, Boston, Massachusetts 02115. An Affirmative Action/Equal Opportunity Employer. W550(A)

CIRMF — GABOON

VETERINARIAN

International Center of Biomedical Research in Franceville — Gaboon is looking for a veterinarian in charge of Higher Primates as well as of other monkeys and usual laboratory animals.

Ten years' experience wished. French language not necessarily needed. Two-year contract renewable will be offered. For details write to our office in Paris CIRMF, 28 boulevard de Grenelle 75015 — Paris. W540(A)

PLANT GENETICIST UNIVERSITY OF FLORIDA

THE DEPARTMENT OF BOTANY

invites applications for a tenure track

ASSISTANT/ASSOCIATE PROFESSOR

position in plant genetics. Applicants with experience in biochemical/molecular/somatic cell genetics are especially encouraged to apply.

The appointee will be expected to establish an active research program, develop and teach a plant genetics course at the advanced undergraduate/graduate level, and participate in the general education biology courses.

Rank and salary will be determined by the experience and qualifications of the appointee.

Send application, a statement of research interests, and three letters of recommendation, before January 31, 1980, to Dr Indra K Vasil, Department of Botany, University of Florida, Gainesville, FL32611, USA. An equal opportunity/affirmative action employer. W555(A)

MEDICAL RESEARCH COUNCIL

UNIT ON THE EXPERIMENTAL PATHOLOGY OF SKIN

(University of Birmingham)

TECHNICIAN

(Temporary Appointment)

A Technician is required to assist in cell separation procedures and biochemical studies of mammalian cell membranes. A degree or similar qualification in biochemistry is essential.

The post is a temporary one for 1 year to a maximum of 18 months. Salary £3,768.

Applictions in writing to Dr H J Yardley, MRC Unit on the Experimental Pathology of Skin, The Medical School, Birmingham B15 2TJ. 3790(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

Applications are invited for the post of Lecturer in Pharmacology. The principal duties of the person appointed will be to teach science students. Preference will be given to applicants with post-graduate experience in physiological pharmacology. Salary scale £4,333 to £8,992 per annum.

Applications, together with the names of three referees, should be received not later than December 17th, 1979, by the Registrar, The University, PO Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref RV/842/N. 3778(A)

Applications are invited for a position at the

EUROPEAN MOLECULAR BIOLOGY LABORATORY

in Heidelberg, Federal Republic of Germany, as

BIOCHEMISTRY TECHNICIAN

The successful applicant will work on the *isolation* and *purification of enzymes* and will be expected to operate a fermenter for bacterial cultures of up to 100 litres.

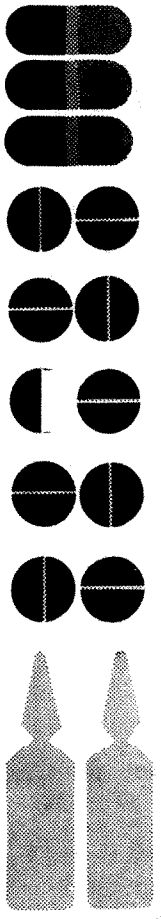
Applicants should have had appropriate technical training and some experience in *enzyme purification* or *nucleic acid biochemistry* is highly desirable. A working knowledge of English is essential.

The salary offered to the successful candidate will be between DM 2200 and DM 2700 monthly, in addition to which certain allowances are payable, depending on personal circumstances.

Please write briefly for an application form, quoting reference 79/18, to:

EMBL, Personnel Section, Postfach 10.2209, D — 6900 Heidelberg, Germany.

W558(A)



Head of Pharmacology Ware

An opportunity has arisen for an experienced pharmacologist to head up a department primarily engaged in research aimed at identifying and developing improved drugs for the treatment of cardiovascular and respiratory disease and disorders of the gastrointestinal tract. You should have a proven scientific understanding of at least one of these areas, a good working knowledge of other scientific disciplines and the ability to lead multi-disciplinary project groups.

You will be expected to make a scientific contribution to existing programmes and initiate new research projects. The Head of Department will also be responsible for the management and administration of the Department and its staff of 70, one third of whom are graduates.

You should be in your mid-thirties, preferably with experience of pharmacological research in an industrial environment.

The post carries a competitive salary and a company car. Other benefits include pension, profit sharing and productivity schemes, and relocation assistance if appropriate to this pleasant river-valley location.

Further details will be sent upon request. Applicants should send a full curriculum vitae in confidence to:

Ian Collins, PhD, Personnel Manager, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. Tel: Ware 3232. 3812(A)

Glaxo Group Research Ltd.

THE UNIVERSITY OF LEEDS

ASTBURY DEPARTMENT OF BIOPHYSICS

Applications are invited for a post of

POSTDOCTORAL RESEARCH FELLOW

to work on a project, supported by the SRC, concerned with the study of molecular conformations and inter-molecular interactions by use of an interactive computer display system. Topics being investigated include the conformation of insulin and drug-receptor interactions. Applicants should have experience in crystallographic studies of biological molecules and in computing. The appointment will be made for a fixed period of up to eleven months.

Salary at an appropriate point on the IA scale for Research and Analogous Staff (£4,333 to £7,521) (under review from 1 October 1979) according to age, qualifications and experience.

Preliminary enquiries about the post will be welcomed by Professor A C T North, Astbury Department of Biophysics.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 42/2. Applications should be submitted as soon as possible. 3803(A)

SYDNEY HOSPITAL

Director of Medical Research

Kanematsu Memorial Institute

Applications are invited from medical graduates with senior degrees and substantial research experience for the position of Director of Medical Research.

Sydney Hospital, the oldest Hospital in Australia, was founded 191 years ago. It is situated in the centre of the City of Sydney and is a Teaching Hospital of the University of Sydney. During the year 1978/79, 18,828 inpatients were admitted to its 476 beds and 250,000 persons attended as outpatients.

The Kanematsu Memorial Institute, an integral part of Sydney Hospital, was established in 1933 and provides a clinical service as well as both clinical and applied research.

The Director will be in charge of the Medical Research Department which has well equipped laboratories, a workshop, an animal house and its own associated wards. The Director may develop fundamental and/or clinical research along any line appropriate to the work of the Hospital.

The salary will be that of a Senior Medical Specialist (at present up to A$39,061). There is also an administrative allowance and a right of limited private practice.

A supporting statement giving information about the Department, the Institute, and the Hospital, as well as details of the conditions of employment and instructions to applicants is available from the undersigned.

Applications close on 1st January, 1980, and should be forwarded to the undersigned in an envelope marked "Director of Medical Research". Further enquiries should be addressed to Chief Executive Officer, Sydney Hospital, Macquarie Street, Sydney, NSW 2000, Australia. 3723(A)

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nature
London
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New York, NY 10020
(212) 765-5758

WORCESTER COLLEGE OF HIGHER EDUCATION

County Council of Hereford and Worcester

LECTURER IN HUMAN PHYSIOLOGY

Applications are invited for the above post from candidates with appropriate first and higher research degrees to lecture in the College Honours degree programmes validated by CNAA. The appointment will be from as early in 1980 as possible.

Candidates should have particular interest and expertise in the field of exercise physiology and will be expected to play a leading role in the development of courses in Human and Social Biology.

The salary will be at an appropriate point in the Lecturer Grade II/Senior Lecturer Scale, £4,542 to £8,325.

Application forms and further particulars may be obtained from The Principal, Worcester College of Higher Education, Henwick Grove, Worcester WR2 6AJ, to whom completed application form should be returned as soon as possible. 3780(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

UNIVERSITY OF SURREY

DEPARTMENT OF HUMAN BIOLOGY AND HEALTH

RESEARCH TECHNICIAN GRADE 5

Required for interesting work in the field of neurobiology. Candidates must be of a good academic and practical standard and be competent to work with the minimum of supervision under the direction of the Reader in Physiology. A small amount of class preparation may also be involved.

If you have a degree or an HNC in Applied Biology, Advanced City & Guilds Laboratory Technicians Certificate or similar, and have several years' laboratory experience we would like to hear from you.

The Department is in a new building in attractive surroundings, adjacent to an historic town with excellent recreational facilities on the campus and in the surrounding area.

Further particulars and application forms for the post may be obtained from the Staff Officer, University of Surrey, Guildford, Surrey, GU2 5XH, or telephone Guildford 71281, ext. 452. 3807(A)

university college of swansea

SENIOR RESEARCH ASSISTANT

Applications are invited for the vacancy of post-doctoral **Senior Research Assistant** in the **Department of Genetics**. Applicants should have graduate experience in microbial genetics and/or biochemistry, and the successful candidate will join an MRC funded project, studying the mechanism of action of tumour promoting chemicals.

The appointment will date from 1 January, 1980, and will be for one year in the first instance, with the possibility of renewal for up to a further two years. The commencing salary will be on a scale up to £4,910 per annum, together with USS/USDPS benefits.

Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by *Friday, 14 December, 1979.* 3798(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF ZOOLOGY

POSTGRADUATE RESEARCH ASSISTANT

Applications are invited from graduates with interests in behavioural ecology to work on a project investigating species ratios in mixed flocks of birds. Research will centre on the influences of species-specific costs and benefits of flocking and feeding strategies on the time budgets of individual birds and the role of time budgeting in producing equilibrium species mixtures.

Applicants must possess a full driving licence and some field work experience would be an advantage. The project is funded by the NERC and the appointment will be made as soon as possible at a starting salary of £3,775.

Applications, together with the names and addresses of two referees, to Dr C J Barnard, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD 3781(A)

GEOLOGY AND GEOPHYSICS

University of California

Berkeley

THE DEPARTMENT OF GEOLOGY AND GEOPHYSICS

anticipates a vacancy for a tenured faculty member at the

ASSOCIATE OR FULL PROFESSOR

level, beginning in 1980. Areas of research interest should be in the fields of geological and geochemical processes responsible for the formation of mineral and energy resources.

For example, organic geochemistry as applied to the origin and thermal evolution of sediments, or isotope geochemistry applied to hydrothermal mineral resources, or the study of stratiform mineral deposits would all be appropriate fields.

Applicants with at least six years post-PhD research experience should send resumes, including the names of three references, to The Chairman, Department of Geology and Geophysics, University of California, Berkeley, Ca 94720, by March 31, 1980.

The University of California is an Affirmative Action employer. W547(A)

SRC

Energy Projects Co-ordinator-

A Senior Appointment

The primary function of the Science Research Council is to sustain high standards of research and post-graduate education in Universities and Polytechnics. Over the past several years, the SRC has been increasingly concerned that its support for energy-related research should be as effective as possible in terms of both the quality of the research and its relevance to national needs. The Council has decided to appoint an Energy Project Co-ordinator to:

i) **act as the agent of the SRC's Energy Committee in simulating a programme of energy research.**
ii) **act as the co-ordinator for the Council's specially promoted programme in Materials and Energy Conservation in the Materials Processing Industries.**
iii) **be responsible to the Director of the Rutherford Laboratory for the Energy Research Support Unit (ERSU) at the Laboratory, and the Energy research carried out there.**
iv) **act as the interface with Government and Industry in the identification of appropriate areas for SRC support.**

Energy research supported by the SRC covers a wide range of disciplines. The person appointed must be able to appreciate the relevance of each type of research and be able to demonstrate the ability to work with colleagues in Universities, Industry and Government. It is expected that the successful applicant will have had several years experience in research relevant to energy and to have held a senior post concerned with Energy in a University, Polytechnic or Industry.

The Co-ordinator will be based at the Rutherford Laboratory. He will have directly available to him the staff of ERSU, and will be able to call upon a wide range of expertise from other staff in the Laboratory.

The appointment will be for a three year term.

The salary will be in the range £14,200 to £17,200 with Superannuation arrangements to be negotiated.

Applicants should send curriculum vitae quoting Ref: VN 860 to Jane Griffiths, Recruitment Office, Building R20, Rutherford & Appleton Laboratories, Chilton, Didcot, Oxfordshire OX11 0QX to whom enquiries should be made.

Closing date for applications is:-

7th December, 1979.

3799(A)

UNIVERSITY OF TORONTO

DEPARTMENT OF GEOLOGY

Applications are invited for the position of

RESEARCH ASSOCIATE

to work with the Ultra-Sensitive Analysis Facility. This facility, incorporating ion microprobe, DC accelerator and mass spectrometer is being set up jointly by the Departments of Physics and Geology. The applicant should have a geological background, competence in physics, and a familiarity with instrumental methods of analysis of rocks and minerals, preferably including mass spectrometry. Duties will be to participate in the establishment of the laboratory, and in the development of geochronological and ultra-low level trace element analytical methods. The appointment will be for a maximum period of three years. Salary will be based on NSERC post-doctoral rates. Send resume and names of two referees to: Prof. D.W. Strangway, Department of Geology, University of Toronto, Toronto, Ontario, M5S 1A1. W542(A)

Ireland

Veterinary Surgeon

required for a position as

Fish Pathologist

in the Department of fisheries & Forestry

Salary: £7,187 – £10,116. Entry up to **£9,014** possible

Essential: Experience in Fish Pathology (research towards a relevant post-graduate degree acceptable).

Upper age limit: 40 years.

For further details and application forms write to:

The Secretary, Civil Service Commission, 1 Lower Grand Canal Street, Dublin 2.

Closing date: 13th December 1979

3789(A)

ESSOCHEM EUROPE INC., located at Machelen, near Brussels, the coordinating headquarters for Esso Chemicals activities in Europe, Africa and the Middle East, has a vacancy for a

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W537A

Universiteit van Amsterdam

Applications are invited for the post of

Reader (m/f) in embryology

in the Medical Faculty of the University of Amsterdam. He/she should have a wide knowledge of the field and will be working in the Laboratory for Anatomy and Embryology of the University. Together with members of the staff of this Laboratory he/she will be responsible for the teaching of embryology to medical and dental students. His/her research interests should be in molecular biological aspects of embryological development. Apart from pursuing his/her own work he/she will be expected to participate in current research in this field in the Laboratory.

Salary scales from Dfl. 6,023.- to Dfl 8,604.- max. per month.

The candidate must be prepared to learn the Dutch language.

Applications (with curriculum vitae and list of publications) should be submitted within 2 weeks to the Secretary of the search committee:

Prof Dr. P. Borst.
Jan Swammerdam Instituut,
afdeling Medische Enzymologie.
1e Const. Huygensstraat 20,
1054 BW Amsterdam, The Netherlands.

Telephone: 020 - 181313, ext. 66.

W557(A)

SCOTTISH SOCIETY FOR RESEARCH IN PLANT BREEDING
PRINCIPAL SCIENTIFIC OFFICER

A vacancy exists in the staff of the Potato Breeding Department at the Scottish Plant Breeding Station for a Potato Breeder in the grade of Principal Scientific Officer.

The person appointed to fill this post will be responsible to the Head of Department for a wide range of potato breeding and associated work carried out by the Department, and will be expected to collaborate with other senior staff members in the up-dating and development of the department's breeding programme. The appointee will be required to lead teams engaged in field work, which will involve travel to trial sites in various parts of the United Kingdom, and to liaise with collaborators in the potato processing industry and in other breeding organisations.

Applicants should be experienced plant breeders with a flair for planning, analysis and interpretation of field trials data, and a facility for collaboration.

Qualifications: 1st or upper 2nd class Honours degree in a biological subject, with considerable relevant post-graduate research experience.

Salary: In the range £7,534 to £9,646 per annum.
The post is superannuable.

Application forms may be obtained from The Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF, to whom completed forms should be returned not later than 7th December 1979. 3727(A)

Public Health Laboratory Service
Central Public Health Laboratory

Microbiologist

A vacancy exists in the **Bacterial Metabolism Research Laboratory** for a recent graduate to undertake a 3 year project supported by the M.R.C. on the use of gas-liquid chromatography for diagnosis of Legionnaires' Disease. The successful candidate will be expected to register for a higher degree.

NHS Whitley Council conditions apply. Starting salary (from 1.1.80) £4,464 plus London Weighting.

Application forms may be obtained from The Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT, Closing date 7 December 1979.

PHLS

3782(A)

DIRECTOR GENERAL

International Service for National Agricultural Research (ISNAR)

The Consultative Group on International Agricultural Research (CGIAR) is an association of official and private aid donors, co-sponsored by FAO, the UNDP and the World Bank. It currently supports 12 major international agricultural research and technical assistance organisations, requiring an annual budget of some US$120 million.

The CGIAR is establishing a new organization, the International Service for National Agricultural Research (ISNAR) with its headquarters in the Netherlands. Its working language will be English. ISNAR will provide assistance to developing countries in strengthening their national agricultural research capacities. It will be concerned with research policy, research planning, research organisation and research management.

Those interested in being considered for the post of Director General, ISNAR, should apply to the German Agency for Technical Cooperation (GTZ) enclosing a current curriculum vitae, by not later than *December 16, 1979.* The successful candidate is likely to have:

relevant experience in organisation and management, preferably in the field of agricultural research;
understanding of conditions in developing countries;
knowledge of and contacts with development assistance organisations and development assistance programs.

GTZ will be able to provide additional information.
ISNAR — Secretariat
c/o German Agency for Technical Cooperation (GTZ)
Postfach 5180, 6236 Eschborn 1
Federal Republic of Germany.

W561(A)

CANCER RESEARCH UNIT, University of Western Ontario, London, Ontario. Positions are available for staff at the Assistant or Associate Professor level (non-tenure track) and for postdoctoral fellows and graduate students. Research is focussed on the areas of cellular and viral DNA replication, nucleic acid metabolism in eukaryotic cells, mRNA cloning, and mechanisms of cell transformation.

The unit is well funded by the National Cancer Institute of Canada and is affiliated with the Department of Biochemistry and the Department of Microbiology and Immunology in the Medical School of the University.

Candidates for a staff position should submit a curriculum vitae, statement of research interests, and the names of three referees to Dr. David T. Denhardt, c/o The Administrative Secretary, Cancer Research Unit, University of Western Ontario, London, Ontario N6A 5B7, Canada. Students should apply for admission through the above mentioned Departments. W544(A)

COLAISTE NA hOLLSCOILE CORCAIGH
UNIVERSITY COLLEGE CORK

BIOCHEMISTRY

Applications are invited for a research position supported by a grant from the National Board for Science and Technology.

The Research project is concerned with the relationship between purine metabolism and various stages of meat production.

Applicants should have either an honours degree in Biochemistry, or an honours degree, with Biochemistry as subsidiary subject. Research experience is desirable but not essential.

The appointment is for one year in the first instance with the possibility of extension for one or more years. Salary will be in accordance with qualifications and experience.

Applications, including a Curriculum Vitae and the names of two referees should be sent to Dr Eric R. Tully, Department of Biochemistry, University College, Lee Maltings, Prospect Row, Cork (Ireland). 3791(A)

Please mention **Nature** when replying to these advertisements

ASSISTANTSHIPS

UNIVERSITY OF OXFORD
DEPARTMENT OF THEORETICAL PHYSICS
POSTDOCTORAL RESEARCH ASSISTANTSHIP IN SOLAR PHYSICS

Applications are invited for a Postdoctoral Research Assistantship supported by the SRC for studies of solar flare and active region physics.

The project will make use of observations to be obtained from the Solar Maximum Mission satellite, and experience in theoretical work associated with the physics of high temperature plasmas, including spectroscopic techniques, would be an advantage.

The appointment would be from October 1, 1980, for a maximum of three years. Salary would be on the appropriate point of the SRC IA scale.

Applications with curriculum vitae and names and addresses of two referees should be sent by December 31st to Dr C Jordan, Department of Theoretical Physics, 1 Keble Road, Oxford OX1 3NP. 3796(P)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF MICROBIOLOGY

Applications are invited for a
POSTDOCTORAL RESEARCH ASSISTANTSHIP
financed by the S.R.C. for work on biochemical and genetic aspects of anaerobic energy metabolism in *E.coli* involving several λ-cloned functions. Tenable for three years.

Starting salary up to £5,199 a year. Applicants should possess or expect soon to possess a PhD in molecular biology, genetics, microbiology or biochemistry.

Applications, including curriculum vitae and names of two referees to Dr J R Guest, Department of Microbiology, the University, Sheffield S10 2TN as soon as possible.

Quote ref: R 384/G 3805(P)

GRANTS and SCHOLARSHIPS

UNIVERSITY OF LONDON
GRANTS FOR RESEARCH

Applications are invited from members of the University and teachers in Schools of the University for grants from the Central Research Fund to assist specific projects of research and for the provision of special materials and apparatus.

Grants are not made for normal maintenance.

Applications must next be received not later than 15 December 1979. Forms of application and further particulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, London WC1E 7HU.

3783(H)

ANNOUNCEMENTS

ANNOUNCEMENT

Cold Spring Harbor Laboratory

COLD SPRING HARBOR, LONG ISLAND, NEW YORK 11724

MEETING ON

THE BIOLOGY OF THE VASCULAR ENDOTHELIAL CELL

MAY 6 to 11, 1980

The main purpose of the meeting is to bring together investigators in the field of endothelial cell biology to discuss new findings in the following areas:

GROWTH AND REGENERATION
- Control of contact inhibition
- Tumor angiogenesis factor
- Defined growth factors & hormones
- Extracellular matrix: effects on proliferation & cell morphogenesis

CELL-CELL INTERACTION
- Tumor cells & vascular endothelium
- Platelets, granulocytes, erythrocytes
- Endothelial cell-smooth muscle cell interaction

METABOLISM
- Prostacyclins, angiotensin, serotonin
- Fibrinolysis, plasminogen activators
- Factor VIII, von Willebrant
- Lipid metabolism, LDL, HDL, chylomicrons
- Lipoprotein lipase

STRUCTURE
- Cytoskeleton
- Cell surface
- Basement membrane, Fibronectin
- Surface specializations
  - junctional complexes, coated vesicles, vesicular transport

Persons interested in attending please contact:

Mrs. Gladys Kist, Meetings Secretary
Cold Spring Harbor Laboratory
P. O. Box 100
Cold Spring Harbor, New York 11724

W548(G)

GRANTS and SCHOLARSHIPS

UNIVERSITY OF WAIKATO
New Zealand

HILARY JOLLY PHD SCHOLARSHIP IN LIMNOLOGY

(Vacancy A79/21)

Under the terms of a bequest from the late Dr V H Jolly, the University of Waikato offers an annual scholarship for doctoral work in limnology, for a period of up to three years. Limnological work in the University is centred in the Biological Sciences Department, where the main emphasis at present is on small dystrophic lakes. Work is in progress on their plankton, benthos and palaeolimnology. Opportunities are available for work in these and other freshwater habitats in the Waikato Valley.

The scholarship will carry remuneration of NZ$4,020 per annum for up to three years. An allowance for expenses incurred during the fellowship of up to NZ$750 in any one year may be available. No financial assistance however will be payable to an appointee in taking up the scholarship.

Further information on conditions of appointment and method of application is available from the Registrar, University of Waikato, Private Bag, Hamilton, New Zealand, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. Enquiries of an academic nature may be addressed to Dr M A Chapman, Reader in Biological Sciences, in the University.

Applications should be in the hands of the Registrar by: *12 January 1980.*

3810(H)

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Nature
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STUDENTSHIPS and FELLOWSHIPS

THE UNIVERSITY OF BIRMINGHAM
FACULTY OF MEDICINE AND DENTISTRY
DEPARTMENT OF PHYSIOLOGY
RESEARCH FELLOW

Applications are invited for a three year MRC financed research fellowship. Salary will be on the scale Research Fellow 1A (£4,333 to £7,521) plus superannuation. Maximum starting salary will be £4,910.

The project involves an electrophysiological analysis of chemo — and baroreceptor afferents to the hypothalmic supraoptic and paraventricular nuclei.

The successful candidate, who will work with Dr M C Harris, should have a PhD, experience with neurophysiological techniques is essential and a knowledge of histochemical technique would be an advantage.

Further information may be obtained from Dr M C Harris.

Applications (three copies) should be sent to the Assistant Registrar, the Medical School, Birmingham B15 2TJ, with a full curriculum vitae and naming three referees by 14th December 1979. 3802(E)

THE UNIVERSITY OF SUSSEX
SCHOOL OF BIOLIGICAL SCIENCES
POSTDOCTORAL RESEARCH FELLOW GRADE 1A

Applications are invited for an SRC financed Postdoctoral Research Fellowship to work in the Schools of Biological and Molecular Sciences on the ecomones which influence the behaviour and physiology of the snail hosts of bilharzia with Dr J D Thomas, Dr F McCapra and other members of an interdisciplinary group working on various aspects of the chemical ecology and biological control of pulmonate snails. The post is tenable until 31st March 1980, with prospects of renewal, to commence as soon as possible.

Research experience in either Biochemistry, Phytochemistry or Physiology, and a broad interest in the field of chemical ecology, would be considered and advantage. Salary on the Grade 1A scale £4333 — £7521 p.a.

Curriculum Vitae and the names of two referees should be sent as soon as possible to Dr J D Thomas, School of Biological Sciences, University of Sussex, Falmer, Brighton from whom further particulars may also be obtained.

3785(E)

THE ROYAL SOCIETY
BRUNO MENDEL FELLOWSHIP

Applications are invited for a Bruno Mendel Fellowship for one year from 1 October, 1980, renewable for one and exceptionally two additional years, from postgraduate candidates normally resident in the United Kingdom, The Netherlands, or Israel, to carry out research in medicine or the biomedical sciences at a university, medical research centre or medical school.

The stipend will normally be at the level of a junior research fellowship appointment in the United Kingdom; additional allowances for travel and superannuation may be provided as appropriate.

Application forms, receivable by 15 January, 1980, and regulations (revised in May 1976) are available from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

3797(E)

UNIVERSITY OF NEWCASTLE UPON TYNE

Applications are invited for the following Fellowships (tenable for two years from October 1980) from persons who have shown themselves able to carry out original research. The value of each Fellowship is £4,333 in the first year and £4,622 in the second year (under review).

SIR JAMES KNOTT FELLOWSHIPS

TWO Fellowships available in any faculty.

LORD ADAMS FELLOWSHIP

ONE Fellowship available for research in any faculty. Preference will be given to candidates who, through family, residence or education, are connected with the former County of Cumberland or whose proposed research has connections with, or will be of benefit to that County.

Further particulars and application forms (which must be returned by 31 December 1979) may be obtained from the Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU. 3784(E)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

in Laser Physics in the Department of Natural Philosophy to work on a C02 TEA laser contract. Previous experience in laser physics and vacuum technology would be an advantage.

The appointment which is on Range 1A of the national salary structure for research and analogous staff, will have a salary within the range £4,333 to £5,777 (under review) with placing according to age, qualifications and experience. USS benefit. The appointment is until 30th September 1984 in the first instance.

Written applications including a curriculum vitae (quoting R44/79) and the names of two referees should be sent as soon as possible to Professor A.L.S. Smith, University of Strathcylde, Department of Natural Philosophy, John Anderson Building, 107 Rottenrow, Glasgow, G4 0NG from whom further details can be obtained. 3792(E)

QUEEN MARY COLLEGE
UNIVERSITY OF LONDON
LASER MEASUREMENT OF BOVINE SPERMATOZOA MOTILITY

Applications are invited for a 2-year Postdoctoral Research Fellowship for work with Dr D A Ross, in conjunction with the Ministry of Agriculture Cattle Breeding Centre, Reading, verifying the viability of routine assessment of samples of frozen semen, using the new fibre optic Doppler anemometer, and to correlate motility measurements with fertility trials.

Candidates with a PhD in Physics or Electrical Engineering and interest in or direct experience with laser anemometry or photon correlation preferred. Initial salary in range (under review) £5073 — £5650 p.a. (including London Allowance).

Please apply, giving age, qualifications, experience and names of 2 referees, to The Secretary, Queen Mary College, Mile End Road, London E1 4NS. 3809(E)

UNIVERSITY OF WARWICK
CASE Studentship — Kinetics of Diazonium Salt Reactions by Fast Reaction Methods

Applications are invited for a three-year SRC (CASE) Research Studentship in the Department of Chemistry and Molecular Sciences, starting 1st January, 1980; and supervised by Dr T J Kemp in collaboration with Dr J Atherton (ICI Organics). The programme will involve a kinetic study of the decomposition and coupling reactions of ArN_2^+ ions using stopped-flow spectrophotometry combined with a rapid-scanning spectrometer which provides complete u.v. — visible spectra in milliseconds.

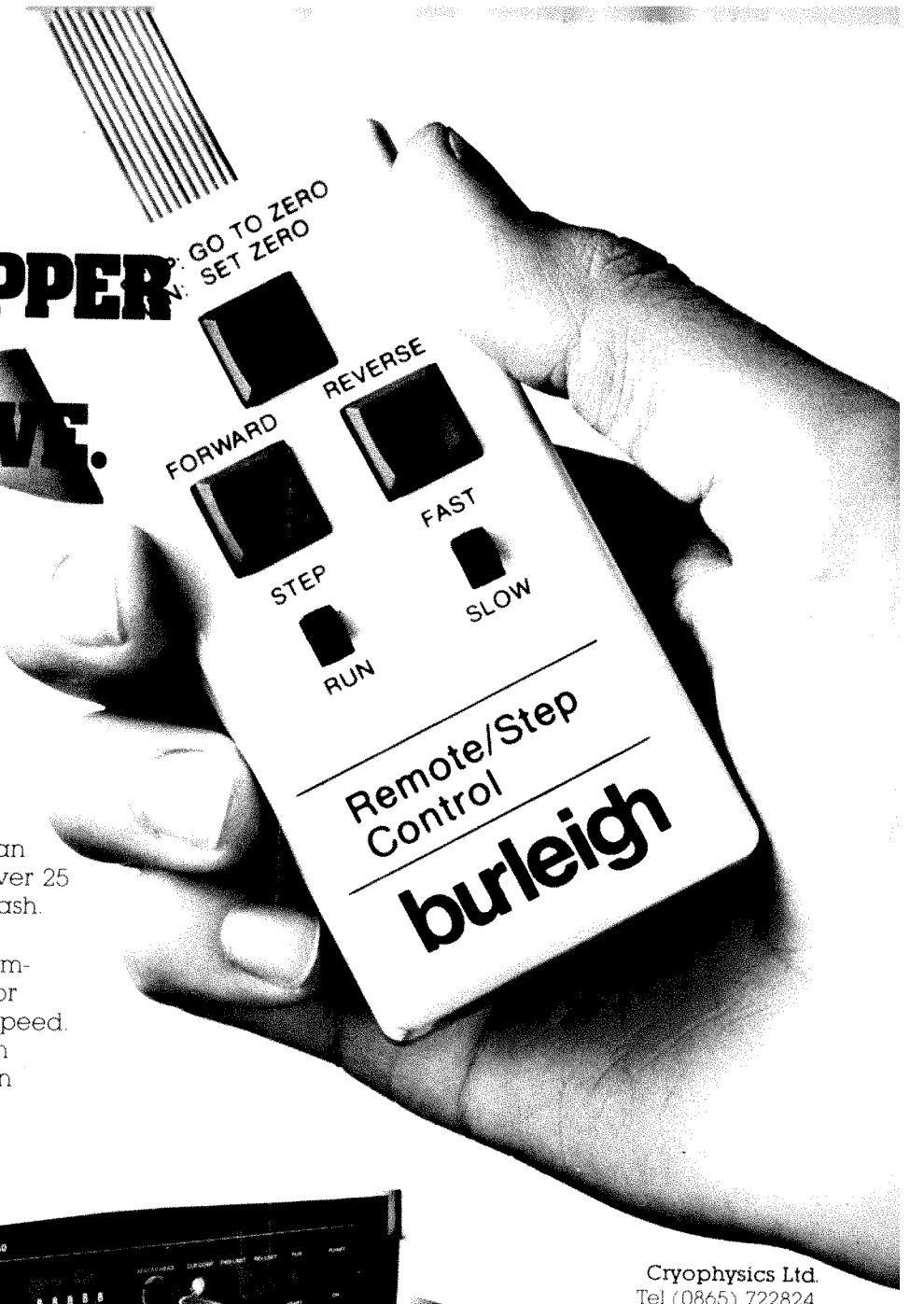
Applicants should hold at least an upper second class honours degree in chemistry. Further details may be obtained from Dr Kemp, Department of Chemistry and Molecular Sciences.

Applications should include a curriculum vitae and the names of two referees and be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No: PG/40/79 by 15th December, 1979.

3801(F)

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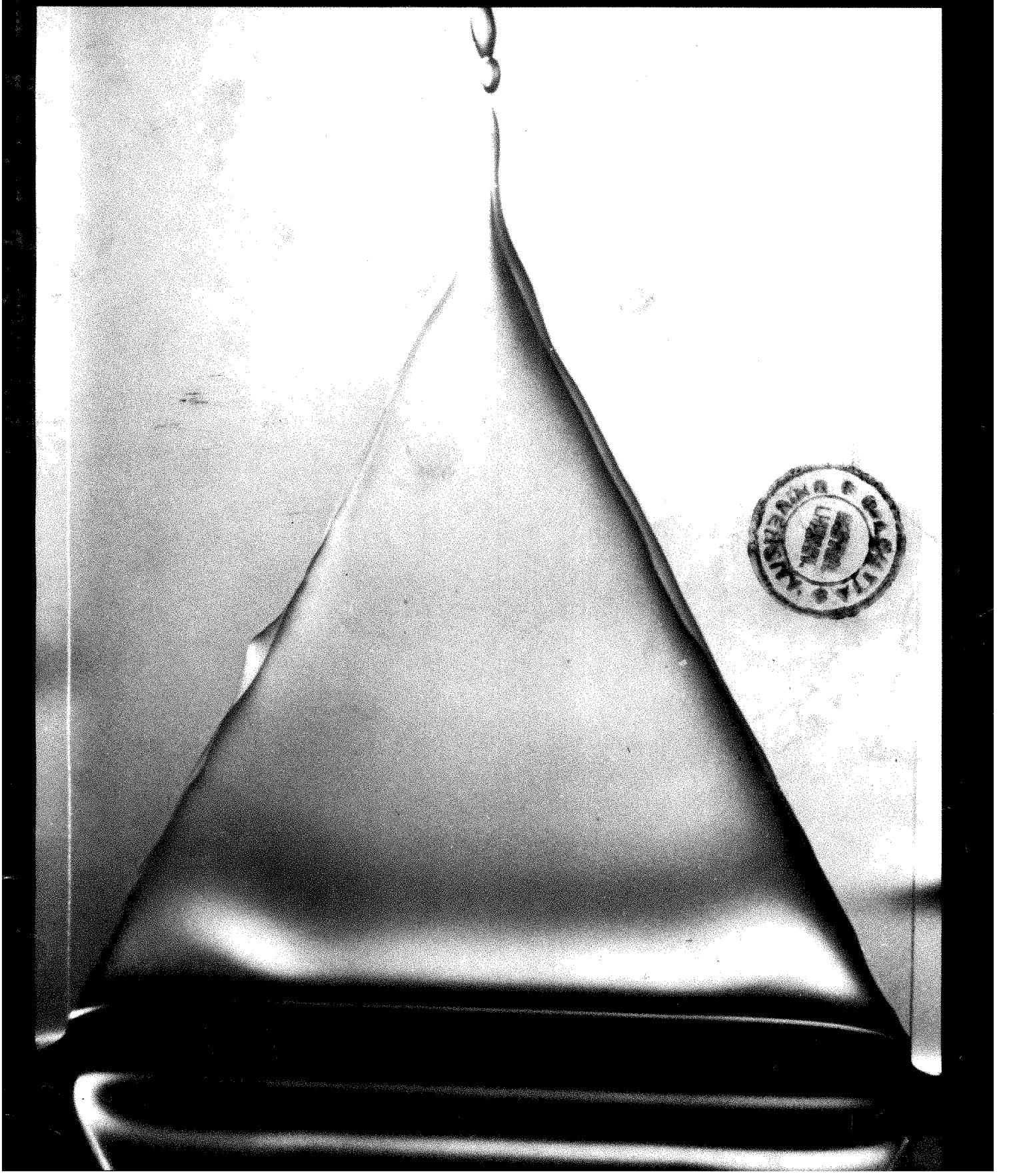
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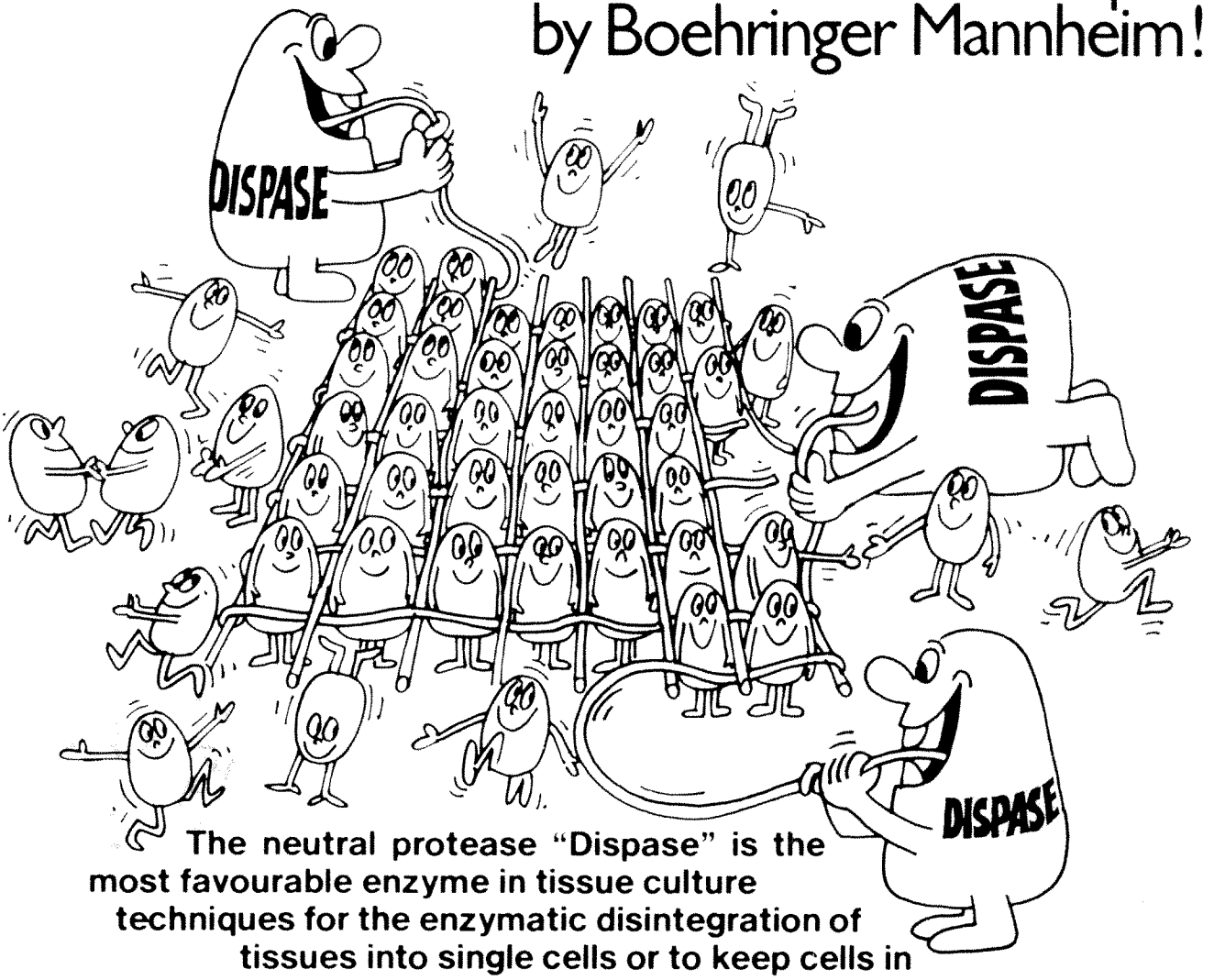
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Cover picture

The sawtooth wetting line formed on a polyester tape being steadily withdrawn from a glycerol/water mixture. This phenomenon is discussed on page 489.

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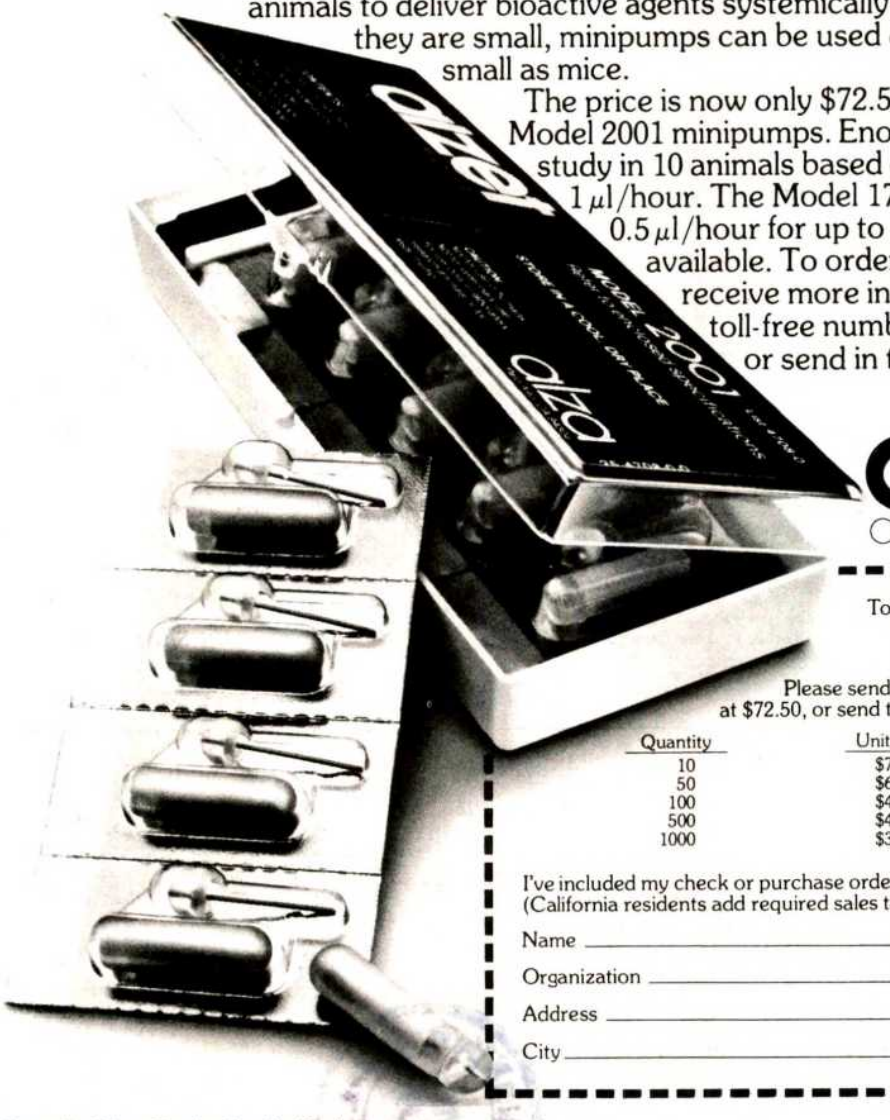
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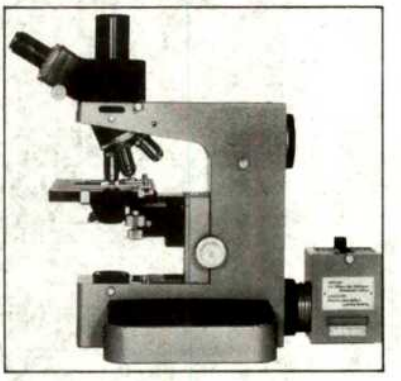
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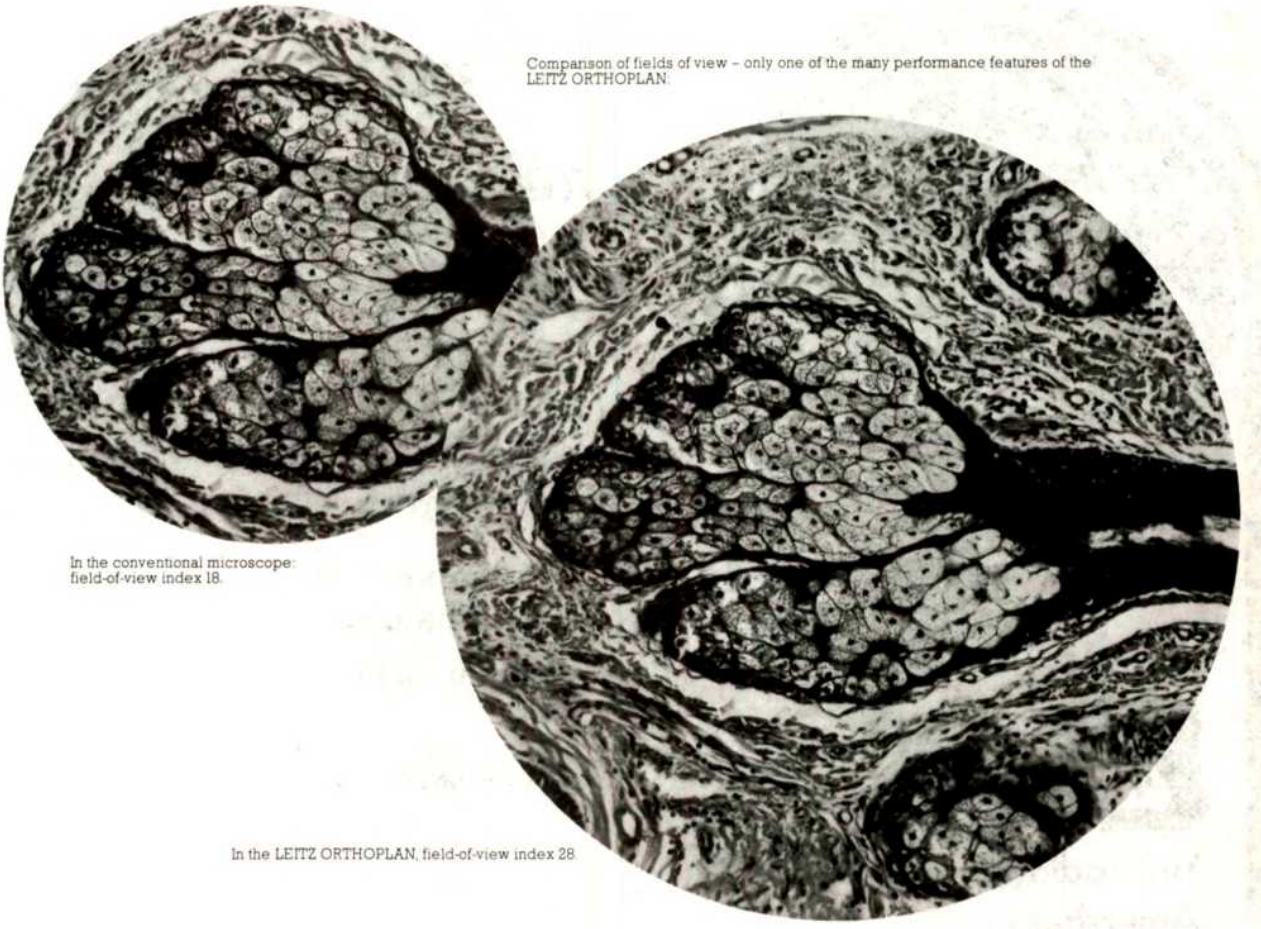
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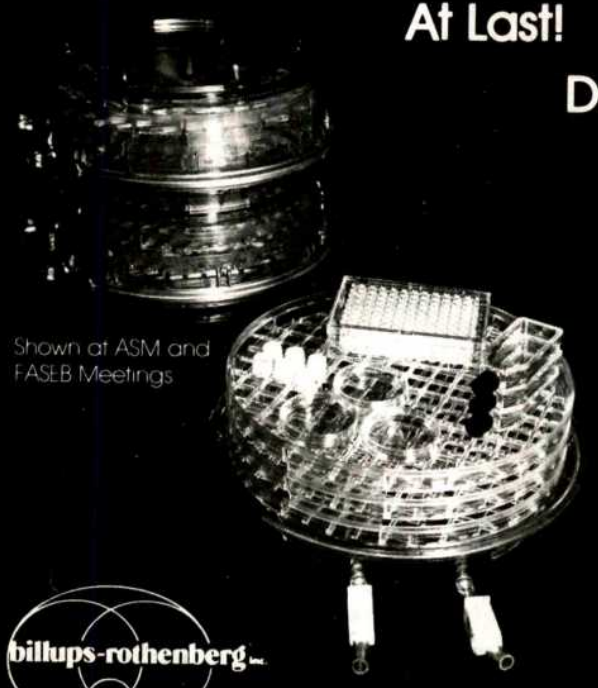
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nature

29 November 1979

Universities are an international heritage

OVERSEAS students in British universities, whether at the undergraduate or postgraduate level, will have to pay more — much more — for their education from next September. The fees will vary from subject to subject but will range from £2,000 to £5,000 per year; at the same time, sources of assistance for students in developing countries, whose governments are unlikely to be able to find the additional sums, are drying up with the decline in scholarships that the British Council can provide on its trimmed budget.

Not surprisingly, in the weeks since the announcement was made arguments about the folly of the increase have been widely aired — particularly those which point to the financial benefits which accrue to Britain from overseas students. Against these are ranged arguments that if British education is all that good, market-place forces will continue to bring overseas students in droves; and if, on the other hand, it comes off badly in international comparisons, then again the market-place will convey a clear message and we will all have to pull our socks up.

The trouble with the market-place argument is that whilst the prospective purchasers of everything from toothpaste to automobiles can at leisure compare costs and assess values, the specific question of which university in which country is likely to provide the best (whatever that means) education for an individual student is almost unanswerable. Decisions are based as much on tradition and hearsay as anything else. Maybe for this reason, good students will continue to be sent to Britain. No-one yet knows. But whatever happens it is foolish to talk of higher education existing in anything like a market-place.

The real concern over students' fees ought to be the implications they convey, not altogether unjustly, of a developing defensiveness in Britain against foreigners taking any advantage whatsoever of the British taxpayer. Traditionally, British governments have recognised the intagible benefits which come from a generous policy towards students — not least that places of learning are an international, not just a national, heritage and that a leavening of overseas students helps keep them that way. If the present government is seen simply to be milking overseas students for what it can get, then, no matter whether enough rich students can be found from overseas to keep the numbers up, the character of Britain's universities will slowly change for the worse.

Monitoring developments in numbers of applicants is not enough. It says nothing about those who have been frightened off by the price from filling in a form. The government needs to take remedial action now, probably in the form of a scholarship fund, to ensure that deserving students from poorer countries get a chance. □

Threshold treaty would bê a mess

PROGRESS these past few months towards a ban on underground nuclear weapons tests has been glacial. The United States, Soviet Union and Britain continue to meet regularly in Geneva to discuss technical matters such as the number of monitoring stations that each country will have, what instruments the stations will contain and so on. But there seems little sense of urgency, despite the obloquy that nuclear powers are bound to encounter during the Non-Proliferation Treaty Review Conference next year if they have failed to deliver on a treaty which has a high symbolic value to non-nuclear states.

The idea that the treaty might be of indefinite duration has long since been abondoned; a short-term ban of three years with no promise whatsoever of renewal is now the most that will ever be negotiated. But even that now shows danger of being eroded. There are rumours that whilst Britain is at present maintaining an official line that a total ban is still the desirable outcome, privately views are being expressed that a much better treaty would be one which kept the concept of a threshold. A figure mentioned is 15 kilotons. Explosions with yields above this would be forbidden, explosions with lower yields would not.

Technically, this sort of treaty would be next to impossible to police properly. At present the United States and Soviet Union have a threshold agreement that neither will fire devices of more than 150 kilotons, and even at this level there have been occasions when serious questions have been asked about whether particular tests have contravened the limit: such are the vagaries of geology. A reduction of this limit by a factor of ten would cause much greater difficulty, because signal levels recorded by various seismic stations around the world would then be closer to the level of the general noise background. As a consequence the error bars that have to be assigned to yield estimates would expand unacceptably; it would be a foolhardy person who was prepared to say that any particular device was, say, 20 kilotons rather than 15. The consequences of so many false alarms are not difficult to imagine.

There are strong political reasons why a comprehensive test ban should be comprehensive. There are equally strong technical ones. □

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US debate heats up over energy conservation strategies

Higher energy prices may encourage more efficient use — but will the poor have to bear an unfair proportion of the costs? **David Dickson** reports



The American poor have little money left for heat or light

THE recent take-over of the US Embassy in Teheran has focused Washington's attention even more sharply on the need to reduce dependence on imported oil. And this in turn has emphasised the gains to be made through measures to increase energy conservation.

Yet the appropriate strategy to achieve energy savings has become a major source of political controversy. At stake is the basic trade-off between energy efficiency and energy equity: if the price of energy is raised to encourage conservation, can this be done without imposing an unfair burden on those who would be proportionately most affected by the higher costs, namely the urban and rural poor for whom energy costs take up to 30 to 40% of the weekly income?

This conflict has been most visible in early sparring between the two main contenders for next year's Democratic presidential nomination. President Carter has made oil price de-control central to his energy strategy, arguing that the price of domestic oil should be allowed to rise to that set by the OPEC countries — and that there should be a 'windfall' profits tax on the oil companies from which the worst effects of the price increase can be mitigated.

In contrast, Senator Edward Kennedy has in recent weeks been arguing strongly against decontrol, suggesting that in current circumstances "it would seriously exacerbate our inflation and produce 'conservation' only as a by-product of a massive recession". Higher prices may in the long run be desirable to encourage economic competition — but in the present economic climate they would be unjust.

Not that the Carter administration has been idle on conservation. Last week, for example, the Department of Energy published proposed building regulations estimated to add 3 to 5% to the costs of new residential and commerical buildings — but to result in energy savings of 20 to 50% through setting overall consumption rates needed to qualify for federal loan guarantees and other benefits.

But after setbacks in other parts of its proposed policy, the administration has recently switched its main focus to energy supply matters, such as the proposed massive programme to develop synthetic fuels. And the conservation ball has been passed back to Congress, where the Senate's Labour and Human Resources Committee is supporting a bill that would provide $2.2 billion within three years to help poor people insulate their homes.

This see-sawing between the administration and Congress reflects the difficulties that surround attempts to devise a strategy for conservation, where the unpredictable effects of policy measures — as well as the complexity of putting them into practice — are even greater than for energy supply decisions.

Indeed it is only relatively recently that the full potential of conservation for reducing energy demand, not only for transportation but also for space heating and other domestic uses, has been accepted in policy circles. And there has been a tendency to lean heavily on the effects of 'price signals' — the expected response of individuals to increased energy costs.

Using such signals, for example, a study prepared two years ago for the National Academy of Sciences' Committee on Nuclear and Alternative Energy Systems (CONAES), produced estimates showing the wide range of energy-use forecasts resulting from different pricing strategies. It predicted, for example, that a four-fold increase in energy costs would lead to energy use remaining approximately constant between 1975 and 2010.

In practice, however, price signals have so far not provided a particularly good guide to the behaviour of car drivers, either in seeking energy-efficient vehicles or in cutting down consumption of petrol. "I am probably less optimistic now about how readily people take to economic signals", Dr John Gibbons, chairman of the NAS panel and now director of the Office of Technology Assessment, said last week.

"I am still convinced that people make rational economic decisions about future choices — but it seems that you still have a lot of social inertia, perhaps because the path to high energy efficiency is complex".

Experience has therefore shown that the relationship between price and consumption is even more elastic than previously thought. This has led to greater consideration of non-price mechanisms, inevitably involving greater federal intervention. At the same time energy problems have become intertwined with broader attempts to cope with inflation.

Here the choice is whether, as Senator Kennedy argues, the government should tackle inflation head-on by trying to keep prices down until the economic situation improves — or to argue that eventually inflation can only be contained by realistic pricing strategies, and that the government's role should be to stimulate the operation of market principles.

"In the long run, it is going to hurt if prices stay down", says Dr Lee Shiffer of the Lawrence Berkeley Laboratory in California, a member of the panel which produced the conservation report for CONAES. "One thing that our study found was that for the majority of consumers, energy standards will only be accepted if . . . the savings are in the same ball-park as the costs."

The panel's calculations show that only when the cost of oil rises to about $30 a barrel do major conservation programmes begin to look attractive. And this strategy is also supported by many environmentalists, who have argued that increased conservation will not only slow the growth of pollution-generating sources, but will also spur the search for more desirable sources of energy, such as solar power.

Such arguments, however, are meeting increasing opposition from community activists and others concerned more directly with social welfare issues. Gar Alperovitz, for example, of the Center for Economic Alternatives in Washington,

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argues that whatever the theoretical principles involved, steps to offset the inflationary impact of increased energy prices are unlikely to prevent the poor from being required to shoulder a disproportionate amount of the burden.

The effects of recent price increases on such groups is illustrated by a survey prepred for the office of consumer affairs of the Department of Energy. This points out that between 1973 and 1977, the median income of poverty-level families increased 34.7%, while the costs of household fuels increased 78%, and has risen even more dramatically since then.

The survey found that for many poor families, food was the first budget item to be cut in order to pay high utility bills. "Since few of them spend much money on anything except housing and food, there is nothing else to eliminate . . . many of them are without heat at all in the winter", the survey says.

If nothing else, current debates have raised a general awareness that energy politics have important equity implications, and cannot be resolved merely by technical arguments. But the tensions between environmentalists and community activists is symptomatic of the disagreement that remains over appropriate strategy.

Many are now demanding a stronger government role in regulating demand, through rationing, heavy energy taxes, and tough conservation standards. They point, for example, to the example of Portland, Oregon, which has recently introduced a statute which requires all home owners to introduce conservation measures if they want to sell or to rent their properties.

"We cannot realistically overcome the inequity of higher energy prices" says one community worker from New York. But with Congress in its current anti-regulatory mood, alternative strategies are unlikely to get very far, and protection for the poor will have to be sought by other routes. □

Canada boosts university research by 32%

THE Canadian government has announced that the budget of the Natural Sciences and Engineering Research Council (NSERC) will be increased by 32% in the financial year 1980-81, to a total of $159.8 million.

The increase is the first of a number of measures expected to be made public in the next few weeks for stimulating Canadian research and development. This is in line with the government's pre-election commitment, reaffirmed two weeks ago by the newly-appointed Minister for Science and Technology, Mr Heward Grafftey, to raise Canadian R & D expenditure from 1% to 2.5% of the gross national product.

The extra money has been provided to the research council, which is responsible for supporting research in Canadian universities, in response to a five-year plan which the council submitted to the government earlier this year.

"We are currently operating with a budget which is 14% lower in real terms than it was ten years ago. This is obviously a very significant increase, although still less than we had asked for", Dr George Macnabb, President of NSERC, told *Nature* last week.

In response to current and projected manpower shortages, the council is planning to introduce a research associate scheme, under which it will provide salary costs and additional research support over a five-year period to post-doctoral scientists at universities prepared to commit 90% of their time to research. About 100 such awards will be made annually.

Another plan is to provide summer support for undergraduates in their second or third year who want to gain research experience. The council is currently working out details on how the extra money for equipment should be distributed. "We are trying to undo the damage of the last decade, and hopefully we can do it in a planned way", says Dr Macnabb.

As for research grants, the precise distribution of awards will not be announced until February, but it is planned that although the major part of the increase will go to basic research, there will be proportionately a larger increase in support for targeted research.

The NSERC'S budget increase has been generally welcomed by Canadian scientists, who in recent months have been lobbying for an end to the decline in federal support for research through groups such as the Canadian Federation of Biological Societies and the Canadian Association of Physicists (CAP).

"The federal commitment to improve support for fundamental research in the universities and to ensure the creation of a healthy and vital university research community is long overdue", Dr Robert Willes of the CFBS commented last week.

Dr John Kucharczyk of CAP, however, warned that scientists still had some reservations. These included: doubts whether the commitment to increased funding would continue for more than one year; doubts whether the provinces and private industry were prepared to provide the support on which the government's strategy depended for research; and concern over the constraints placed on the NSERC by the fact that less than half its requested budget increase had been accepted.

The Canadian government is expected to announce shortly increased funding for both the Medical Research Council and the Social Science Research Council. And the budget to be presented to Parliament in December is likely to include details of tax incentives offered to industry to invest in research and development.

David Dickson

US drops back in biology and maths

THE total number of US papers appearing in the world's leading scientific publications fell by 6% between 1973 and 1977, according to figures released in Washingon last week by the National Science Board, the policy arm of the National Science Foundation.

US prominence dropped particularly in biology and mathematics. In biology, the net number of papers by US scientists in the Science Citation Index's two thousand most influential journals fell by 11%, representing a drop from 46 to 42% of the total biology papers. In mathematics, the proportion of US-authored papers fell from 48 to 41% over the four years.

This decline, which was mirrored but not quite so sharply in almost all scientific disciplines, was partly offset by an increase in the average frequency with which papers published were cited. In 1973 the rate of citation of US papers was 28% above what would have been expected on purely numerical grounds. By 1977 this had risen to 30%.

These figures, based on data gathered by Computer Horizons Inc. from the Institute for Scientific Information, are published in *Science Indicators 1978*, presented to Congress last week by President Carter as the annual report of the National Science Board.

Despite frequent statements to the contrary, the report paints a relatively healthy picture of US research and development, suggesting that an apparent decline in international terms may be largely the result of countries such as Japan and West Germany catching up with the US's previously unchallenged position.

"Among the two major types of research activity — basic and applied — a period of modest growth has persisted for longer than any time since the late 1960s" the report says. Even in industry, it states, expenditure on basic research grew from $719 million in 1975 to $975 million in 1978, an increase in constant dollars of 14% over the years.

The report points out that the "phenomenal growth" which has taken place in energy research and development since 1974 — an occurrence which, contrary to expectations, it says did not take place in other industrialised countries — has slowed down, although perhaps only temporarily. But a shift from military to civilian R & D within the federal budget seem to have been reversed in the last two years. □

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Defoliant companies can be sued

A US federal judge ruled last week that five chemical companies which produced the defoliant "agent orange" can be sued by Vietnam veterans. The veterans claim that they were injured by exposure to the chemical, and that their children were deformed. Agent Orange contains the defoliant 2,4,5-T, suspected of containing the toxic contaminant dioxin; it was extensively used in Vietnam between 1962 and 1971.

The veterans want a trust fund to be set up and financed by the five chemical companies — Dow Chemical, Monsanto, Thompson Hayward Chemical Company, Hercules Inc, and Diamond Shamrock — for the benefit of those who, they claim, suffered as a direct or indirect result of exposure to the defoliant. According to the veteran's attorney, claims against the companies could be $40,000 million.

The companies had contended that the 362 veterans are entitled only to veteran's medical benefits, and that the suits should be tried individually in state courts. However, Judge George C Pratt ruled that a suit could be brought against the companies.

The US Defense Department was accused last week of severely underestimating the number of soldiers exposed to Agent Orange during operations in Vietnam. Although the Pentagon has until recently said that all combat zones sprayed with the defoliant were free of army personnel, a report issued last Saturday by the General Accounting Office, the investigatory arm of Congress, said that at least 5,900 marines were within a third of a mile of the areas while or immediately after they were being sprayed. The GAO also says that a further 16,000 marines were within a third of a mile of the sprayed areas within four weeks of the spraying, and it called the army's contention that troops did not enter the area until six weeks afterwards as "inaccurate". In a letter to Senator Charles Percy who had requested the GAO investigation, the Department of Defense admitted that "the chances that ground troops were exposed to herbicide Orange are higher than DOD previously acknowledged". □

UK radical scientists ten years on

THE 10th anniversary of the founding of the British Society for Social Responsibility in Science was celebrated in London last week by a congenial gathering of 200 activists representing a span of 60 years of experience of radical and socialist approaches to science. Five speakers analysed the past and present successes of the "largest single grouping of radical scientists in Europe".

Maurice Wilkins, president of the society, evoked the social atmosphere requiring new approaches to the practice of science by emphasising the turbulent state of world affairs. "Things have changed a very great deal in a very short time. We have seen a continuous disintegration and degeneration of our institutions in modern society over the past 50 years. This disintegration has certain advantages since it increases the possibility of getting outside our conditions. It is difficult to overestimate how strong our social conditioning is and BSSRS correctly has been concerned to show that science cannot escape from these conditions."

Joseph Needham, who is as close to a patron saint as is possible for a political organisation to have, presented a unique mixture of Christianity and socialism with a blessing for BSSRS' past and future work. He affirmed his conviction in the achievements of socialism. "Socialism has been an outstanding success in China which was absolutely medieval in character in the 1940s. I think we have to recognise that India will never get anywhere unless it follows the Chinese way."

Strong continuities exist between the issues of the 30s science movement and those of present groups. Racism, science in war, organising scientific workers, developing links with left wing movements, demystifying science to a larger audience and the position of women in science are all issues that lie outside mainstream "establishment science". One of the great achievments of the 30s movement according to Needham was the campaign leading to the eventual construction of the Anderson air-raid shelters prior to the second world war. "We knew it was important and possible and in spite of government indifference we shamed them into doing it."

Speakers, Dorothy Griffiths, Jonathan Rosenhead and Mike Cooley outlined BSSRS recent history and immediate future plans. In terms of analysis, the group has moved away from the "use-abuse" model of science and technology and is concentrating on developing its understanding of how science is thoroughly integrated into the social fabric through its employment in profitable economic production, its use in social control technology and its use as "a justification for social inequality based on class, race and sex." New activities include a formal Women's Caucus, a working group to criticise the UK government's proposal to count and predict the size of Britain's black population, particularly the proposed introduction of a question on racial origin in the 1981 census, and a working group that will provide an information service to trade unions on the likely impact of the proposed introduction of microprocessor based technologies in particular workplaces. **Joe Schwartz**

London meeting calls for end to psychiatric abuse

THE recent meeting in London of the World Psychiatric Association focused attention, once again, on the use of psychiatry for political repression. Although the 1977 Honolulu Congress of the WPA formally condemned psychiatric abuse, there has so far been little concrete action from its special "Committee to Review the Abuse of Psychiatry". *Ad hoc* pickets at the meeting and Amnesty International in its formal letter to the WPA executive, stressed the need for firm and expeditious action from the Review Committee.

The Amnesty appeal stressed the "consistent pattern" of psychiatric abuse in Romania and the Soviet Union, particularly the latter. Details of over 40 recent Soviet cases were appended, with "symptoms" ranging from Seventh Day Adventism (Eugenius Darguzes) to membership of an unofficial Marxist discussion group (Alehsandr Skobov) and from organising an independent trade union (Vladimir Klebanov) to proposing Academician Andrei Sakharov as a candidate for the election to the Supreme Soviet (Vladimir Tsurikov). In spite of the recent criticism of "Snezhnevskiism" (*Nature* 11 October, page 419) Snezhnevskii-type diagnoses of "sluggish schizophrenia" continue — as does the "treatment" of dissent by massive doses of neuroleptic drugs.

Perhaps one of the most telling comments on psychiatric abuse is the fact that the occasional transfer of a dissident such as M Kukobaka (charged with "anti-Soviet slander") from psychiatric hospital to trial and prison is viewed by Soviet human rights campaigners as a qualified triumph. **Vera Rich**

Galileo formally reprieved

POPE John Paul II has called for a formal reversal of the Catholic Church's verdict on Galileo, who in 1633 was condemned by the Roman inquisition and placed under house arrest for his heliocentric theory of the solar system. The Pope was addressing a special session of the Vatican's Pontifical Academy of Sciences, held to mark the centenary of the birth of Albert Einstein.

He quoted the Pastoral Constitution *Gaudium et Spes* No 36/2 of the second Vatican Council, which deplores "certain habits of mind sometimes found too among Christians, which do not sufficiently attend to the rightful independence of science. The arguments and conflicts which they spark lead many minds to conclude that faith and science are mutually opposed". These words, said the Pope, related in particular to the Galileo case. □

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News in brief

US waives air pollution rule for coal-burning power plant: The US Environmental Protection Agency announced last week that it would temporarily waive air pollution rules to allow New England's largest power plant — in Somerset, Massachusetts — to convert from oil to coal two years earlier than planned. The utility had been ordered to convert to coal in 1977 by the Federal Energy Administration, and although the EPA had agreed to the conversion, the need to install pollution-control equipment meant that the conversion was initially planned to be phased in between 1982 and 1984. However the EPA has agreed to a temporary waiver of the pollution rule covering particulate emissions.

Converting most power plants to coal is a key element of the Carter Administration's energy programme, and EPA deputy administrator Barbara Blum said that the waiver, which will not involve the violation of atmospheric air quality standards protecting public health, was an "important milestone" towards energy independence.

British Council of Churches urges abolition of UK nuclear missiles: By a vote of 54-19, the semi-annual Assembly of the British Council of Churches has urged the UK government to abandon its plans to replace Polaris submarine missiles with newer nuclear missile systems. The Assembly resolved that "non-replacement by the UK of its present nuclear strategic deterrent (the Polaris missile system) would strengthen moves for nuclear non-proliferation and urges Her Majesty's government to take a decision to this effect."

In its presentation of the resolution, the Council's Division of International Affairs, argued that "unfavourable consequences" could be minimised by careful implementation of the decision and that there is "no hope of reversing the nuclear arms race until a nuclear weapons state is willing to demonstrate that the arms spiral can go down as well as up. Britain now has a unique opportunity to demonstrate that nuclear non-proliferation is a realistic rather than Utopian goal." At present the UK has four nuclear submarines each armed with 16 missiles which were obtained "at rather advantageous prices" from the US in 1962. Each missile contains three UK manufactured warheads. The US now has 2,141 missiles capable of delivering 11,000 warheads and the Soviet Union has 2,582 missiles with about 5000 warheads.

Jacob criticises new social darwinists: French Nobel Laureate François Jacob has vigorously attacked the "ideological distortions" of biology propagated by social darwinists, racists and others in an attempt to "justify certain models of society". M Jacob is a coauthor of a recent report for the French government on bioengineering and has written a major five part series on biology for the French daily *Le Monde*. In these articles he takes severely to task those who, like the French "new right" or certain sociobiologists, would see in genetic diversity a racial weakness or an excuse to justify inequality and injustice. Indeed, says Jacob, human potential depends on this diversity. "Any effort that aims at the homogenization of the biological properties of individuals — either by hoping to 'ameliorate' them through eugenics or by seeking to encourage one attribute like an aptitude for mathematics or for running — would be biologically suicidal and socially absurd".

Refusnik charged with plagiarism: Dr Vladimir Kislik, a leading figure among Soviet Jewish "refusnik" scientists has been threatened with a charge of plagiarism and breach of copyright. Dr Kislik, whose work has been largely in radiation physics, especially radiation defects in solids, applied for an imigration visa for Israel, in 1973. Permission was refused, on the grounds that radiation physics was "secret", and he was obliged to leave his post at the Kiev Physics Institute. Since then he has worked as a nightwatchman, and as bus-ticket seller, and has been subject to constant police surveillance. Deprived of any way of continuing his scientific career at home, he managed to send abroad a paper on the kinetics of helium release from irradiated samples of austenite steels, which was published in the *Journal of Nuclear Materials* in 1978.

Recently Dr Kislik was warned that a charge is being prepared against him under article 136 of the Ukrainian penal code which deals with plagiarism and patent infringements. This appears to be in connection with a thermokinetic method of He analysis which he developed while at the Kiev Physics Institute, and with the unauthorised publication of his paper abroad.

This is the first time that the smear of plagiarism has been raised against any refusnik scientist, and appears to form part of a new "pre-Olympic" drive against all forms of dissent and nonconformity. Ironically, Kislik himself could have a strong case against the authorities under the said Article 136. As with Academician Veniamin Levich, after being driven out of academic life, Kislik's name "disappeared" from citation of his published papers.

India's wild life projects under spy cloud: Many of the wild life projects which India is currently carrying out in collaboration with western countries have come under suspicion of being convenient covers for espionage in the far-flung, strategic borderland of India. The Public Accounts Committee of Parliament, after investigating various collaborative wild life schemes in India, has implied that a project on migratory birds is involved with the perfecting of biological weapons. The US is the guilty partner in this case and the work undertaken in the swampy, sprawling Sunderban, known for its variety of migratory birds, has come under severe public criticism.



"All the beat produced was three KGB, one MI5 and a brace of CIA!"

Another project that has become a focal-point of public controversy is the "pheasant project" undertaken in collaboration with the International World Pheasant Association of the UK. It involves the selective reintroduction of pheasant in the foothills of the Himalayas in the states of Uttar Pradesh, Himachal Pradesh and Jammu and Kashmir. It is feared that the foreign collaborators might use the pheasant project as a camouflage for spying in the sensitive borderland.

B Radhakrishna Rao

Four thousand in French protest march: One of the largest demonstrations by scientific and technical researchers in the last ten years marched through Paris on 22 November. Responding to a national call from all the trade unions in the scientific sector to demonstrate opposition to the government's reorganisation plan for the CNRS, scientists and workers from this organisation were joined by colleagues representing the medical, agricultural and overseas research institutions of France in a march from the Sorbonne to the Presidential palace. A delegation handed in a protest against a government reform they feel was pushed through without consultation and which will lead to a dismantlement of French research, overdependency on the criterion of profitability and an increasing level of job insecurity for the country's scientists.

Correction: In "UK to tighten asbestos control", (1 November, page 5) crocidolite should replace chrysotile in the second paragraph of the second column. The first sentence of the fourth paragraph in that column should refer to the 0.5 fibre per ml^{-1} limit for amosite.

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France faces a cold winter

The discovery of cracks in the tubing of most French nuclear reactors has lead to a strong call for a national debate on nuclear energy. **Jim Ritter** outlines recent events.

TROUBLES, indeed, come not singly but in armies; and over the past two months France's nuclear programme has found itself under siege by a formidable host of them. The leaks of primary coolant which have, for the moment, shut down half of the country's present generation of nuclear reactors, and the discovery of cracks in the tubing of almost all reactors, either under construction or in operation, promises a cold winter for the French people and a series of agonising reappraisals for their government.

The decision was taken in 1969, under the last government of Pierre Messmer, to create an 'all nuclear' electrical power industry. The development programme was placed in the hands of the state electrical generating board, Electricité de France (EDF) which opted for a two-tier, short-term and long-term programme. The short-term programme involves the building of 900 MW pressurised water reactors under licence from Westinghouse. The long term programme involves the development of fast breeder reactors. The present problems lie in the PWR systems.

By the end of August 1979 the EDF had succeeded in putting into operation six PWR reactors, two at Fessenheim, and four at Bugey near Lyons, the latter alone supplying some 10% of present French electrical generating capacity. Three more PWRs at Gravelines, at Tricastin and at Dampierre were due to be integrated into the network by the end of this year, with another 26 to be added over the next five years (some of these to be of 1300 MW capacity).

But on 22 September the two principal French trade unions, the CFDT Confederation Française Démocratique de Travaille (CFDT) and the Confederation Générale de Travaille (CGT), sent an open letter to M André Giraud, the Minister for Industry, signalling the existence of numerous cracks in key components of the cooling system of almost all reactors then under construction and, most likely, in those already in service.

The PWR construction firm Framatome had known of these faults since mid-1978 although neither it nor the EDF had made this information public. This the unions meant to remedy. The government's own report appeared on 3 October and was in substantial agreement with that issued by the unions.

Part of the trouble lies in a metal plate, 3.5 m in diameter and 0.53 m thick, used in the steam generation system between the primary and secondary coolants. The plate is pierced by 6776 holes, each 22.22 mm wide and protected against corrosion by a 10 mm thick layer of Inconel alloy. The alloy is deposited in two stages, both involving the heating of the element to 200°C, generating great thermal stress. It is during the second of these processes, the formation of the Inconel-Inconel bond, that the problem arises. Each plate develops between 30 and 200 cracks from 15 to 20 mm long and 6-8 mm deep. Worse still, it was discovered that similar cracks appeared during the fabrication of the six tubes which connect the reactor chamber with the steam generator, tubes which not only carry the hot, pressurised primary water but also support the entire 400 tonne weight of the reactor itself.

Although these cracks are not dangerous at this stage, Framatone experiments show that they tend to enlarge under the temperature transients of normal operating conditions, leading to possible component failure within 4-6 years, a failure which clearly would have catastrophic consequences involving the total loss of primary coolant.

What then can be done? The steam generating plates are replaceable, though not those in the six reactors already in operation. For these, and for the entry and exit primary tubes, repairs will have to be effected *in situ*. But ambient radiation levels rule out the use of human labour and as yet undeveloped automatic, remote techniques will have to be used. Indeed, the very possibility of such cracks occurring was not even considered in any of the preliminary safety calculations. The only other possibility is permanent shutdown after five years which, given the present cost of 3000 million FF for each reactor, would render the whole nuclear programme economically unfeasible.

These were the facts made public by both government and unions. Along with their report the unions demanded that the loading of fuel elements at Gravelines, Tricastin and Dampierre be halted until further checks were made. The EDF replied 11 days later, in issuing their report, that the loading would continue on schedule. The CGT and CFDT then called out their members in a five day strike which ended in the government's agreement to a cessation of loading and a new investigation. While this second investigation was underway the Ministry for Industry launched a counter-offensive in the media. On 10 October a ministry official said that the government had instituted its investigation long before the unions' announcement, while government spokesman M Marcel Boiteux said during a television interview that "where (cracks) have been found they are not serious; where they might be serious they have not yet been found".

But further trouble had already arisen. On 10 October there was a leak of primary coolant at one of the Bugey reactors, forcing a shutdown. Now under severe pressure, the EDF announced a blanket censorship on any further information on the nuclear programme; all further statements were to be subject to government clearance. In response a petition which had been circulating, calling for a national debate on nuclear energy and the suspension of all work on nuclear reactors until the conclusion of that debate, rapidly gained sponsors and signatures. The discovery of further leaks at two other Bugey plants and the announcement by the EDF on 24 October of their decision to resume loading at Gravelines (followed by a similar decision for Tricastin though not yet for Dampierre) brought things to a head.

The hitherto united trade union front broke. The CFDT, which like the Socialist Party opposes the present form of the nuclear programme, called for a strike at Gravelines to prevent loading. But the CGT, which along with the Communist Party supports an all-nuclear policy, accepted the EDF decision. Since the CGT was the union of the majority of nuclear technicians the CFDT call was heeded by a comparatively small number of workers. Even so, the loading at Gravelines and Tricastin has had to proceed under police protection with technicians brought in from outside the EDF, an unprecedented situation even in security conscious France.

The impact of the national petition for a national nuclear debate, is not yet known. Public opinion polls before the announcement of the cracks showed pro-nuclear opinion ranging from 53% to 62% with an opposition between 24% and 37%. During recent union elections within EDF, however, the anti-nuclear CFDT gained 2.5% while all other groups, including the pro-nuclear CGT, lost ground. Since this swing now gives the CFDT a majority position at Gravelines and Tricastin, any govenrment decision to start up these reactors after loading is completed in a month's time may be expected to spark off further protest.

Meanwhile, with three of its six operating reactors closed down, the EDF is facing difficult choices this winter. It has already called upon industries to help produce part of their electrical needs themselves, and it cannot avoid unpopular power economies in the domestic sector in the months ahead. With the further delays in the commissioning of new PWRs attendant upon the discovery of structural faults, the vulnerability of an 'all-nuclear' policy has become more evident. □

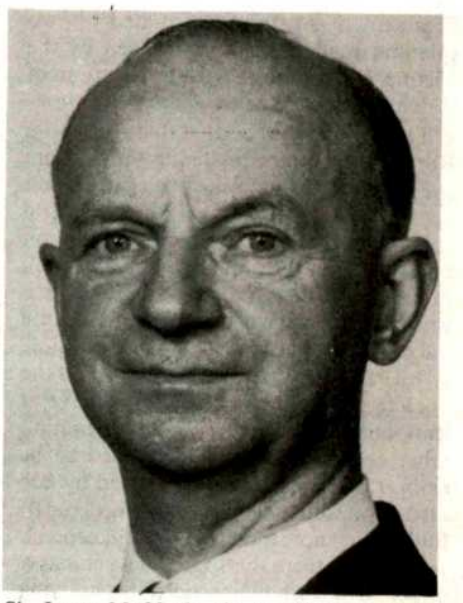
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Development: where are the real experts?

This year's expensive jamboree of United Nations conferences on science and technology for development yielded hollow phrases and little else, writes **Sir Ieuan Maddock.** Funds would be better directed to small teams of trouble-shooters who have direct experience of real problems, such as flood control in Bangladesh or health care in Tanzania; the teams would make "blunt appraisals" and recommend practical actions, building up a library of useful prescriptions.



Sir Ieuan Maddock: the jet-setters must be firmly pushed aside

IF noble phrases, robust restatement of the obvious and general amiability of colleagues is the formula for peace on earth and universal prosperity then the sumposium organised by the United Nations' Advisory Committee on the Application of Science and Technology to Development (ACAST) must indeed be regarded as a historic event.

"The New World Economic Order", "Immediate and Total Disarmament", "Free and Open Flow of all Scientific Information", "Global Mobilisation of all the World's Scientists" and many more such phrases crackled and thundered around the chandeliers of the Festsaale of Vienna's magnificent Hoffburg like a Wagnerian overture. The facts that these noble objectives were very far from being achievable, and that none of the world's scientists had even an inkling that they had been mobilised (by whom? for what?) did not deter the faithful. The existence of the Iron curtain, the troubles of the Middle East, the problems of South East Asia, the stresses in Africa, the conflicts in Central and Southern America, the agonies of Northern Ireland and the economic ills of so many countries were brushed aside. Noble declarations were good for the soul.

Down from Olympus

When ultimately the delegates agreed to clamber down from the top of Mount Olympus they proved to be disappointingly mortal. After turning their collective wisdom to such subjects as food and agriculture, health, natural resources, transport, population, and energy, a series of improvised, working groups produced conclusions which were banal in the extreme. Nothing was said which had not already been said far more competently in a multitude of conferences on the same fashionable subjects many times before. The level of originality can be judged by a few quotes chosen at random as follows:

●On natural resources: "Recommends to government that priorities be set for mineral and energy resources, water resources, marine resources and other natural resources in their development policies". "The multidisciplinary nature of the concerned science and technology requires technical cross-sectoral mechanisms for cooperation and co-ordination".

●On transport: "That the integrated policy and planning for transport must be evolved in developing countries, taking into consideration population growth and movement, land use, urban and rural development, agriculture and food production, . . . research and development and comparative study of the development of transport on the international scene in order to avoid errors of the past".

●On communication: "More attention should be given to the value of telecommunications as one of the important instruments for development. The various forms of telecommunications and their usefulness should be investigated. For example radio broadcasts should not be underestimated".

●On population: "Recommend an increase in real terms in the investment in reproductive research and contraceptive development and adoption". "Because population is both a component and beneficiary of development it is recommended that attention be paid to the creation of development models which feature the dynamics of population".

●On human settlements: "Generally there is need for strengthening the scientific, technological and administrative capability for human settlements planning, policies, strategies and implementation to meet the basic needs of the people. This includes re-orientation of architecture, engineering, planning and management of human settlements".

●On environment: "The scientific and technological community is urged to give increased emphasis to interdisciplinarity as an operational concept for tackling the complex land-use problems of ecosystem management".

●On energy: "All possible types of primary energy resources need to be developed including hydrocarbon, coal, nuclear, hydro and solar. When one of these sources is developed even by a few countries it will release non-renewable resources like hydrocarbon for other countries. From this point of view development of coal and nuclear energy appears to be necessary".

●On industrialisation: "These relate to the enormous technological developments of the developed countries as well as the accumulated problems such as unemployment, low productivity, unequal income distribution in the developing countries, posing major challenges to the technological and industrial developments".

Even the strongest digestion tended to become debilitated on a constant diet of this kind of stuff. If one felt prompted to make a comment based on practical realities, one is inevitably felt a bit of a spoilsport. Why diminish the heady delights of playing God.

I came to the conclusion that the ACAST conference was just about the least effective I had ever encountered — that is until I experienced the United Nations Conference on Science and Technology for Development: UNCSTD.

UNCSTD was held in a giant sports hall with some two hundred individual delegations each with its own table, six delegates' chairs, translation equipment and microphone — the minimum kit, it seems, of any serious minded participating country. Several of the larger nations had 'supporters clubs' seated on the sloping galleries around the floor. Each delegation was allotted fifteen minutes to make a statement and these were interspersed by statements from sundry United Nations organisations and a sprinkling of acknowledged non-governmental organisations (NGOs). After the initial ceremonial opening by the Secretary General of the United Nations and by the

Sir Ieuan Maddock is Secretary of the British Association for the Advancement of Science. He represented the Royal Society at the UNCSTD and ACAST conferences. This article has been submitted to the Council of the Royal Society, and it is now published with the Council's approval.

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President of Austria, punctuated by two splendid renderings of Beethoven by the Vienna Philharmonic, the procession of statement-makers began.

The distinguished delegate from Ruritania would be invited to the rostrum (we were all 'distinguished delegates') and each would make an almost identical speech. This process started around noon on the first Monday and was still proceeding late on Tuesday evening over a week later. One wit commented that the only worthwhile statement in the whole series had been made by Beethoven on the opening day. It was obligatory to have at least one person sitting at the delegation table — although the hall tended to be embarrassingly sparsely populated by late afternoon, and so there was a process of finding a succession of 'watchkeepers'. Junior staff from the appropriate embassy or academics who chanced to be studying in Vienna were pressed in service.

Action in the corridors

Inevitably most of the action occurred in the corridors and more particularly in the 'groups'. There was the 'Group of 77' which represented an initial gathering of 77 less developed nations (there were by now over 120). Then there was the EEC, the Eastern European Group, and the Western European and other Countries Group (known as 'WEOC'), an Asian Group, an Islamic Group and so on and so on. These were constantly meeting, determining 'positions', negotiating between groups, and colliding with each other in a manner that could have provided a dozen plots for C P Snow. It was small wonder that the only outcome of this much publicised meeting was to be a weak soup of half-boiled ideas and heavily spiced polemics.

Much of the trouble arose from the skew distribution of the interests and outlook of the people who served in the preparatory phase and also the main conference. At UNCSTD the delegates were drawn almost exclusively from the ranks of academia, of national or international research institutes, of bureaucracies and of politics.

It was a remarkable defect of the structure of the delegation that there were so few people present with any direct experience of the application of technology. Why were names like Ford, Siemens, Ericson, Hoechst, Roche, Heinz, Mitsubishi, Massey-Ferguson, General Electric, Brown Boveri, Unilever, and Shell absent? Where were the hospital superintendents, the forest planters, the dam and bridge builders and sewerage plant constructors? Indeed water and sewerage received hardly a mention throughout the conference, presumably because these were hardly fit subjects for eminent scientists to be found to be discussing. Why were consulting control experts absent from this conference? Where were craft-guilds (the analogue of London City and Guilds) the technician training boards (such as EITB in the UK) the inspectorate and the specification bodies (BSI in the UK)? Why were there no farmers, fishery managers, food packaging experts, food inspectorates or catering managers to be seen let alone heard? Should not those with direct responsibility for and experience of operating railways, urban roadways, electricity and gas utilities, ports and harbours, fire fighting and ambulance services or telephone networks be at the forefront of the endeavour to harness science and technology for development?

But the whole conference had been conceived and executed by those who so blinded by the pyrotechnics of research that they failed to see, or to admit to seeing that there were others far more relevant and more experienced than themselves for the task in hand.

Although several speakers pointed to the essentially long time scale between even successful research and its ultimate impact on society on a significant scale, the emphasis on research continued. To point out that there was already one billion people on the planet living at the survival threshold and that this number would at least double and possibly treble in the next twenty years — a mere thousand weeks — could not deflect the research enthusiasts from their course. Indeed it was clear that some of the less developed countries thought that, despite the inescapable validity of the points made, the developed countries were raising these points only wilfully to impede the development of the less fortunate countries.

There was the persistent impression that we were dealing with a 'global R&D fund' and that somehow the developed countries had seized 97% of this. The fact that the R&D figure was nothing more than the aggregate of a great mass of individual and unrelated decisions seemed to be difficult to convey. That economic and social order was a prerequisite and not a consequence of a high R&D expenditure was only vaguely understood.

I feel convinced that this intellectual gap would not have been present had the composition of the people attending both ACAST and UNCSTD been different. If the core of the delegates had been drawn from those with direct responsibility for and experience of *applying* technology then the community of purpose would have bridged the gap of semantics or of doctines. Of the delegates less than 10% could be clearly identified as people with direct industrial responsibility and experience.

The real experts

Alas! the problems of harnessing the fruits of science and technology to meet the needs of the less developed nations remains. It is a sad thought that in the two years that it has taken to prepare the UNCSTD conference at least one hundred million people have been added to the ranks of the starving and the diseased. It is pointless continuing to hold giant congresses on this or that aspect of the subject and thus providing fodder to that intellectual jet-set who thrive on a menu of agenda drafting and resolution passing. The problem is too urgent and too serious to be left in such hands. All whose who control the aid funds (which in total are quite large if they could be efficiently deployed) should cease to support them.

Instead, small teams of *real* experts, experienced in the technical, human and economic aspects of each subject area, should be brought together. If it is flooding in Bangladesh that is to be discussed, let flood control experts supported by economists, and management experts be brought together to make a long visit to Bangladesh to discuss matters with those directly concerned, give seminars, inspect facilities (including education and training) and to understand the local and cultural constraints. Let such teams write precise and, where necessary, blunt appraisals in cooperation with the local experts and authorities and let them prescribe a plan of action. If it is the health problems of Tanzania, the housing of Peru or communications in Lapland, let the same general procedure be followed. In this way a library of prescriptions would be built up from which both specific and general action programmes would be derived. Above all a network of effective human links would be established between those who are responsible at the national or local level and those who have access to the relevant technologies.

$50 million meeting

Such a scheme would cost money and take time — but the UNCSTD conference took two years to prepare and is reputed to have cost fifty million dollars. The same amount of money deployed over the same time period in the way described above would already have yielded a great fund of exact knowledge on which current and future programmes would be based. The asset would be accumulating as the mosaic of local pictures built up.

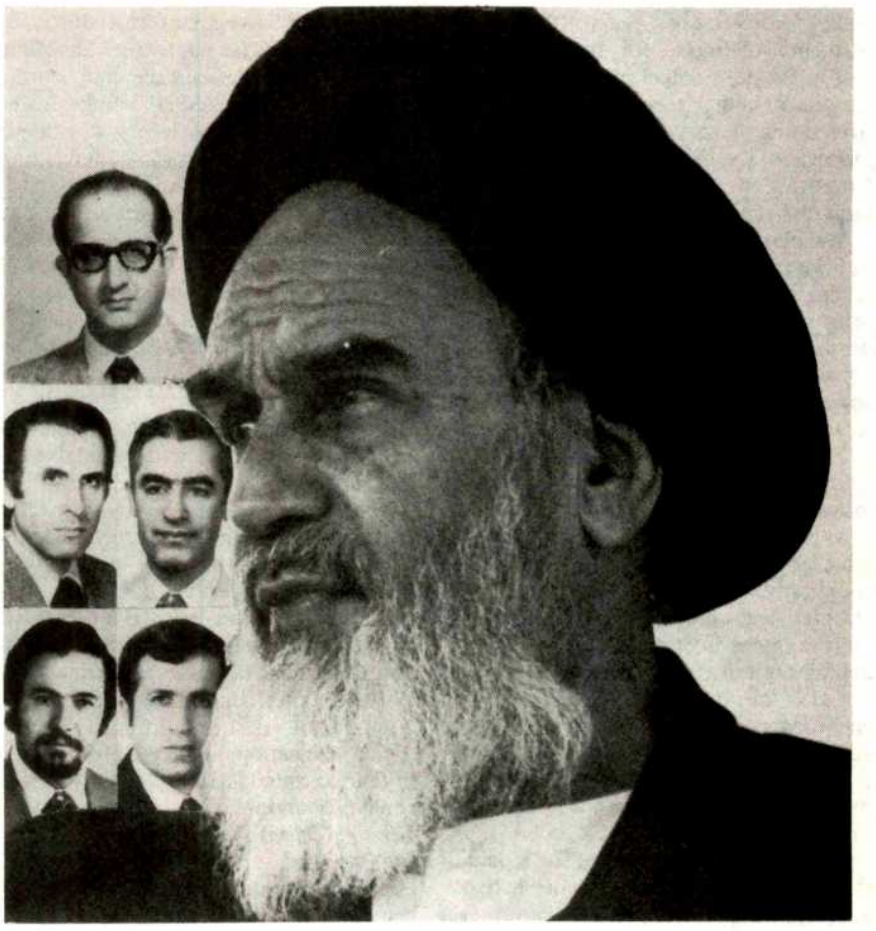
It would be easy just to accept their conference as an agreeable if expensive frolic and to excuse it as the price which has to be paid in the struggle to establish some kind of a world society. Time and circumstances do not permit this kind of tolerant cynicism. With one billion people already at the starvation level and the certainty that this number will treble before the end of the century, it is time for action rather than for conferences. The jet-setters must be firmly pushed aside, they should receive no further encouragement by financial grants or assistance in kind and the task must be steered towards those who are realistic and expeditious. There is no shortage of these if only they are given the opportunity and the support. □

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Scientific thinking behind Khomeini

Hidden within what appears to the West to be an increasingly anarchic Iranian revolution lie a number of young thinkers and ideologues who are planning a new science and technology for Iran. Bitterly critical of the past, they foresee a science which would respect basic human needs and Islamic values. **Zia Sardar,** an enthusiast for their cause, spoke to them



Right: Ayatollah Khomeini and Iran's science policy makers from top and l to r: F Darvish, A Safai, M N Bahadori, M Roohi, A Barzegar.

How will Iran use science and technology to achieve its aims? And what is the role of science and technology in the realisation of an Islamic vision? "We take it for granted that science and technology are a means to an end, and not an end in themselves", says Dr Mahdi Nejat-Bahadori, Professor of Mechanical Engineering at the University of Shiraz and a member of the sub-committee of the Revolutionary Council on Planning in Science and Education. "So we must ask: what ends do we seek?"

"What we have agreed to do", says Dr Bahadori, "is to redefine development as a compound of economic, scientific, technological and social actions that take the society closer to God. And this is used as a guiding principle in all our planning". Within this principle, activities that promote development will be encouraged. But certain scientific and technological activities are too complex and their impact on society cannot be easily appreciated. "For this reason", says Dr Bahadori, "we want all societal changes to be slow, after due consideration and with full consent of the society. We will not commit ourselves to a particular science and technology policy without full consideration of its impact on the society, debate and discussion by our people of it and of its place in our development goals."

Dr Bahadori emphasises that the Iranian revolution is not an economic revolution. "We are not aiming to have a prosperous or a consumer society. Or to further the interests of a particular class or group. Our aim is to elevate human values and our science policy will reflect this aim in all its manifestations".

Dr Ali Barzegar, Deputy Minister of Higher Education argues that a science and technology which elevates human values and the principles of Islam cannot be imported. "They have to be Iranian through and through", he says. "We are not attracted towards big, conspicuous projects with grandiose goals. We are more interested in small, more humble and humane projects that do not get out of control and that utilise our abilities and resources and develop local capability. We want to use science and technology to meet our basic needs and, as these needs are changing, our planning will be continuous. The important thing to realise is that if you do not want to create a consumer society, or chase excessive profits, production becomes a second order determinant. And you can concentrate on quality".

Within the new scheme indigenous research will become very important. Dr M Zirakzadeh, the former deputy minister responsible for scientific research (now replaced by Dr Syed Mohammad Mahdi Jafari), says that universities, government bodies and private industry will be encourged to tackle local problems and offer viable solutions. Financial incentives will be provided. "But the allocation of funds will be on the basis of projects and their importance to Iran, and not on the basis of organisation and research centres", he says. At present there are some 120 research centres in Iran but, according to Dr Zirakzadeh, more than half of them have either been transferring technology or using foreign consultants to get their job done. "Most of these", says Dr Zirakzadeh, "will either be closed or reorganised on a more appropriate basis."

The old National Research Council, which before the revolution was a special committee of the Ministry of Science under the chairmanship of Queen Farah, has been retained with a new mandate to reorganise and revitalise local scientific research. Fields singled out include agriculture, cottage industry, public health and alternative sources of energy, and the development of links with industry and research bodies.

While the new Ministry of Science (a reorganisation of the old science ministry) has been working on a new science policy, a sub-committee of the Revolutionary Council has been working on the new Development Plan. This plan marks a radical change from conventional development planning, just as the Iranian definition of development differs sharply from the commonly accepted ideas.

It is a sophisticated plan with a 12 year horizon, split into three phases. After a two year transition period, the plan proper begins to unfold. It will operate within fixed limits but there will be considerable scope for change and adjustment. Each phase will start at a presidential mid-term so that changes in details can only be made when a particular government has been in

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power for at least two years. "While there is plenty of scope for rethinking and readjustment, changes cannot be abrupt; and the planning process is continuous and over-riding," says Dr Ataollah Safai, Director of the Centre for National Spatial Planning of the Plan and Budget Organisation.

The plan is based on current predictions of both the needs and requirements of Iran and its resources and potential twelve years after the revolution; and it assumes that during this time oil revenues will decrease considerably. It aims to take Iranian society closer to Islam, and has several strategies running in parallel.

The Development Plan concentrates on four areas. First, the fundamental sector of industry, where the emphasis is put on private small cottage industry. Medium-scale industry will be operated on a cooperative basis with the government and essential heavy industry will be run entirely by the government. "The economy is neither capitalistic nor socialistic", says Dr Safai. "Private industry will be encouraged but monopoly will not be allowed. The state will not interfere with the freedom of the individual nor will it have total control over the means of production."

Second, considerable attention is paid to the distribution of economic activity. Strategies for de-urbanisation and rural development are recommended, and a balance in economic activity is sought between the national, regional and local levels. There will be separate programmes for urban, rural and regional systems.

The third area covered is the development of traditional economic sectors — such as agriculture, industry and services within the context of an Islamic society. Both agriculture and cottage industry are given equal emphasis, but services are limited to a level where they do not interfere with the spiritual and cultural values of society. According to Dr Bahadori, the plan will meet all the basic needs of the citizen while at the same time checking materialism. Finally there is an area covering the environment and the creation of a capacity for future expansion, which are intended to create a physical and spiritual environment conducive to the Islamic way of life.

In the area of science policy, the plan emphasises creativity and originality. Dr Safai says "an environment that encourages thinking, with maximum freedom and inducement for originality and creativity — this is our survival imperative". The plan condemns blind imitation of the Western scientific establishment, and envisages the expansion and reorganisation of the educational system to meet the educational needs of Iran.

For education, the country will be organised into regions which will then receive individual attention. This, says Dr Farrokh Darvish, Director of the Institute for Planning in Science and Education, is already happening. Education in Iran has been mainly a paper chase for better jobs and promotions. "Now we are emphasising education which is geared to regional needs", he says.

Although the Iranian educational system faces many new problems such as an acute shortage of teachers, administrators and text books (most curricula have been changed in content or in emphasis) most of its problems are typical of the Third World: irrelevance to social needs, over-emphasis on higher education and on paper qualification.

Although the new Development Plan has been finalised, it awaits presentation to and approval by the Revolutionary Council. Pending its start, the government has cancelled all technology transfer projects, the nuclear energy programme and heavy industry schemes and has reduced the budgets of the existing educational, scientific and research organisations and frozen all expansions that were planned before the revolution. This has created a certain amount of temporary unemployment and ill feeling in industry and education. But, according to Dr Barzagar there is little else that can be done until the society has direction and the plan comes into operation.

The policies put forward so far in the pre-plan period, have been mostly negative however, and some of them have created an uneasy atmosphere. There is also a real danger of over-reaction to the Shah's extreme policies. The pool of technical manpower created under the Shah is now a major asset which must not be forced into self-exile by over-zealous revenge seekers. The revolutionary leadership should try to preserve all of Iran's human resources.

Most of all, the revolutionary leadership must ensure that balance and clear thinking dominates its policies: zeal can sometimes blind reason and humanity. If Iran can avoid this trap it could become a model for all those who seek to build a society on human and spiritual grounds. □

The multinationals come under scrutiny

THERE is considerable ill feeling in Iran against the American and European multinationals and business concerns. "They have plundered Iran without any ethical or moral consideration", says Dr Barzegar. The sales techniques used in Iran, and no doubt they are now being used in other Third World countries, were a balanced compound of Skinnerian Psychology and gangsterism, he says. "Most of the contracts signed with the multinationals by the Shah are ruthlessly one sided".

There are many examples of such joint ventures and technology transfer agreements. In one case, the Shah agreed that General Motors should set up a subsidiary in Iran with the parent company holding 45% of the capital in the US. The agreement stipulated that all key managerial positions, including managing director, treasurer, sales manager and supply manager would be held by American citizens for an indefinite period and that the US parent company would charge the Iranian company a managerial fee and an engineering fee equal to 15% of the cost of machinery.

Even the track record of Scandinavian companies (Scandinavia is considered to have liberal policies towards Third World problems) is poor. For example, consider the case of the Iranian Ball-bearing Company, established in 1969 as an $8.6 million joint venture with the Swedish firm SKF. Even when the Iranian plant began production, SKF continued to import ball-bearings into Iran. As the local product lacked quality, the Iranian Ball-bearing Company suffered tremendous losses and had to reduce its capital by half. SKF, however, made substantial profits.

Iran's dealings with Electronic Data Systems (ESD) are also notorious. Under the Shah, an imaginary demand for sophisticated computers was created. For example, Dr Shojaeddin Sheikholeslamzadeh, Minister of Health and Welfare under the Shah, was trying to implement a mad drive "to have computer terminals in every village in the country, even the remotest, which will let us know immediately a person is ill, so that a central computer can notify the authorities who will send a helicopter to transfer the patient to the nearest hospital". Similar logic was being followed in the Plan and Budget Organisation, the Social Security Organisation, Iran Air, and other government as well as private organisations.

The EDS signed a contract with the Social Security Organisation to computerise its operations. The company supplied a number of packages, without the necessary manpower. None of the packages, however, could meet the needs of the Social Security Organisation. EDS then asked for its contract to be extended from the original three years to five years and the extension was agreed. After four years and $80 million paid out, it became clear to the Shah's regime that EDS had come to stay permanently. Shortly after the firm quadrupled its original demand, with the project still less than one-third complete, Iran had its revolution.

The firm has now sued the Iranian government for breach of contract and the remainder of its money. The action has been taken in the United States and ESD has succeeded in securing the confiscation of the Iranian government's property in the US.

The nuclear programme of Iran under

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the Shah is another example where the Iranians feel they have been exploited by the multinationals. The programme envisaged a network of 23 reactors, supplying 42% of Iran's electricity needs by 1994. Before the revolution, work on four nuclear plants had started and a further 19 were being negotiated.

According to Mr Mansoor Roohi, Vice-President of the Iranian Atomic Energy Organisation (IAEO) the contracts signed for the four nuclear power plants, two to be built by the German company Kraftwerk-union, an engineering unit of Siemens, and two by the French company Framatome, were "remarkably one sided". He claims that the IAEO were warned not to interfere with the work of the foreign companies in case they used this as an excuse for delaying the work. Framatome charged $2.3 billion to build two pressurised water reactors of 900 megawatt near Ahwaz on the Karun river and Krafstwerkunion charged $3.2 billion for two plants of 1,200 megawatts near Bushehr on the Persian Gulf.

According to the IAEO, in order to meet the demands of its nuclear programme, the Iranian government purchased vast quantities of uranium at inflated prices, and directed it to Germany for enrichment. Once enriched, the uranium had to be stored in Germany till 1994, or until it would be needed by Iran. This had been going on since 1974. The Iranian government paid Germany both for enriching its uranium and storing it.

By 1978 the cost of both projects had more than doubled. 60-70% of work on the German plants had been completed, while the sites for the French plants were still being prepared. When regular payments stopped during the revolution, both companies pulled out. They are now threatening legal action.

Iran has decided not to continue with construction of the four nuclear plants and with plans for the remaining 19. A 50-man special commission concluded that the German company had either failed to fulfill all its undertakings or had done shoddy work.

For example, 400 Iranians were sent to be trained in Germany. The commission claimed that they were given the most rudimentary training not much above the technician level. It also considered that the $7 billion sum demanded by the German company to complete the project was unrealistic and that even the $3.3 billion already paid out was grossly unfair for the work done. Sadeq Tabataba'i, Deputy Prime Minister in the Bazargan government, made it clear that "Iran has no intention of paying the compensation".

Meanwhile, the IAEO is rethinking the long-term energy policy of Iran. "We had to abandon the nuclear energy programme because it was designed to keep us in the hands of the foreign companies for decades. Although we are looking into other sources of energy such as solar energy and hydro-electric power, we have not completely ruled out the nuclear option", says Roohi.

Iran is continuing some of its nuclear research and training programmes and a small power plant of 20-30 megawatts will be kept in operation, so that it can retain some nuclear option for the next 10-12 years by maintaining a certain amount of indigenous capability and manpower. However, Roohi thinks that for the "next decade or so, the time needed for us to explore other alternatives and come up with a new, more appropriate energy strategy, Iran will rely largely on its oil to meet its energy needs." □

Iran faces an environmental crisis

DR Taghi Ebtekar, the newly appointed director of the Department of Environment, says that urgent action is necessary to stop further despoilation of the environment. According to Dr Ebtekar, the Shah's economic policies of rapid industrialisation without any consideration for the environment, as well as rapid urbanisation and a growing population, have resulted in alterations to and destruction of many ecosystems.

Complete river systems, such as those of the rivers Ziandrond and Anzali (formerly Phalavi) in the Caspian, are under severe threat from industrial wastes and emissions. Hard detergents from industrial plants have done serious damage to marine life. The Caspian sea is now heavily polluted with "dangerously high levels of DDT, mercury and other heavy metals". "A recent study carried out after the revolution in the northern part of Iran, reveals that the level of DDT and mercury in mothers' milk is up to five times higher than the accepted critical level", says Ebtekar. The Caspian forests, "unique among the temperate forests of the world" are also in great danger.

Vegetation in the northern part of Tehran, he says, is under heavily polluted water and in Ferozabad natural vegetation is swamped by sewage. Air in Tehran contains up to four times the permitted levels of particulates. In Shiraz and Isfahan, air pollution is attacking cultural monuments and threatening the world renowned Iranian architectural heritage.

Soil erosion and soil pollution are also major worries for the department. In the steppes of western Asia, for example,



Traffic pollution is a major problem.

enormous areas are turning to desert. The situation has become so serious, according to Ebtekar, that unless measures are taken to halt and reverse the process, there will be total loss of vegetation over wide sectors with consequent erosion, floods and dust-storms. In other areas, soil, a scarce and precious commodity in Iran, is being polluted by pesticides, heavy metals and fertilisers.

Ebtekar blames the Shah's economic policies for much of the urban pollution. For example, "the Shah promoted private transport and ignored public transport. As a result we have almost 1.2 million cars in a city of 5 million people. Over 80% of urban pollution is the result of exhaust fumes". The Department of Environment has persuaded the government to introduce new, more comfortable buses and to expand the bus routes. It is planning legislation to limit the use of private vehicles and heavily leaded and poor quality petrol. Iranian vehicles will also require "certain devices" to eliminate hydro-carbon and carbon monoxide fumes.

Legislation is also being prepared to enforce appropriate waste-treatment facilities in polluting industries. Sets of National Emission Standards have been developed for application to most industries throughout the country. The department also plans a wholesale conversion to natural gas both for industrial and domestic fuel. Conversion had already started in Tehran before the revolution and will soon be extended to Shiraz and Isfahan.

The department administers 62 reserves for protection of the floral and faunal resources of Iran, constituting one of the most comprehensive programmes for nature conservation in Asia and the Middle East. A number of endangered species are protected in accordance with the wildlife regulations. Of the animals in this category, only the Caspian Tiger (*Felis tigris*) is genuinely endangered, possibly being extinct in Iran. A number of other species, such as Asiatic Black Bear (*Selenarctos thibetanus*) and two species of gazelle and Onager, once believed to be extinct, are now considered safe.

Conservation and protection of wildlife and natural environment are dictated by Islamic law, says Ebtekar. As such, conservation is being promoted at the popular level. "We are conserving our natural resources for the people", he says. "The people must have access to our wildlife, wetlands and other natural resources. We do not want to isolate conservation areas and preserve them as museum objects. Neither will we limit access to conservation areas to certain privileged classes. Everyone will have access to conservation areas as long as conservation laws are obeyed". □

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correspondence

Three Mile Island not "a major disaster"

SIR, — I am writing to comment on the article in your issue of (8 November, page 120) on the Three Mile Island report. In the heading you refer to the accident as "a major disaster". In the text you write "the worst accident in the history of the US nuclear programme". The reader might think there must have been a large number of people killed and injured. In fact there were no deaths and no injuries except psychological. Estimates of the long-term consequences have suggested that a few (less than 10) additional cancers may ultimately develop in the population exposed, an effect likely to be far outweighed by the cancers caused by an extra packet of cigarettes smoked by the same population, or the additional traffic accidents due to the upheaval. How about deaths from bronchitis due to pollution of the air from coal-fired power stations and factories?

A journal of the standing of *Nature* should not be a vehicle of sensational reporting. Many of the recent comments in your columns have shown a total lack of sense of proportion in that the dangers of radiation are never put in the context of the risks we all accept in daily life, nor of the risks attached to not using nuclear power. An irrational fear of radiation has been built up by the popular press and other media; serious journals such as *Nature* should take a more rational and responsible attitude.

Yours faithfully,

D.K. BEWLEY

Medical Research Council, London, UK.

Scurrilous comments belie Egyptian-Israeli cooperation

SIR, — It was difficult to understand the relevance of Dr Galal's comments (11 October, page 424) on the article by Z Sardar (2 August, page 350). The hostile views expressed by the author were in complete defiance of the brave and new approach of Egypt's President Sadat. In spite of Dr Galal's comments I hope that not all the scientific community in Egypt share his views.

It seems that during the twenty years he has been science editor of *Al Ahram*, Dr Galal has adopted the political approaches, but has failed to overcome the psychological gap to which President Sadat has referred many times. What is the relevance of Dr Galal's remarks on "Israeli arrogance in science and technology" to the potential benefit to scientific cooperation? Where did he get his impression of the "superiority complex" of Israeli scientists; or where did he get the idea that Israeli scientists regard their Egyptian colleagues as incompetents who need teaching by us?

I would like to remind Dr Galal that Israeli science has suffered no less than Egyptian science from the continuous wars, and his scurrilous statement on the "colossal funds and privileges that Israel is getting from rich Jewish communities" is far from being "objective, realistic and frank". The money that was given to Israel has been spent to absorb hundreds of thousands of refugees from all over the world, mainly from Arab countries. Was the money from rich oil-producing Arab countries used to improve the condition of the Palestinian refugees? They were kept for more than twenty years in the most miserable conditions and used as a political football. Is that not depriving the Palestinians of their right to live as human beings? The Palestinian problem will be solved only when the Palestinian policy makers stop aiming at the destruction of Israel and recognise the right of the Israelis to live in their homeland. I hope that the Egyptian people, including the scientific community, now agree with their President and recognise the State of Israel, in spite of the problems that still remain to be solved.

Were this to be so, I feel sure that true cooperation between scientists from Israel, Egypt an the world can be achieved, to the benefit of all the peoples of the area.

Yours faithfully,

Z. BEN-ISHAI

Technion, Haifa, Israel.

Antijewishism

SIR, — The style of the letter from Mr Salah Galal (11 October, page 424) is perhaps appropriate for *Al Ahram*, but for *Nature* it seems to be unusual. Though some of the Israeli scientists may have superiority complexes or may be arrogant, the supposition of a national superiority complex and arrogancy of Israeli scientists in general is nothing else than antijewishism. Thus, disregarding their demagogic content, the arguments and statements of Mr Galal have a diminished value because of their motivation by his obviously inherent antijew emotions.

Yours faithfully,

LAJOS ERNST,

Freie Universität Berlin, West Germany.

Distorted interviews in East Germany

SIR, — When I attended the meeting of the Federation of European Biochemical Societies (FEBS) in July 1978 in Dresden, I was invited to give an interview to the Press. This was conducted in German and recorded.

Now, in November 1979 I am surprised to receive "with thanks for my collaboration" a book with contributions to mark the thirtieth anniversary of the GDR (*Of the Birth and Growth of the GDR*, Zeit im Bild, Dresden) in which my question-and-answer interview has been included in the form of a 500-word statement in English. The translation has resulted in a eulogy of the GDR and of GDR scientists, which in its exaggeration is ludicrous. The poetic licence of the translation has caused subtle, and some not so subtle, changes; but when — to give but one example — I am quoted as referring to "a secure existence free from worries of unemployment", I am absolutely certain that I said that I was not competent to answer questions on full employment in the GDR.

I do not blame the colleagues who organised the FEBS meeting in Dresden, of which I still retain a happy memory, but would counsel visitors to the GDR to learn from my experience.

Yours faithfully,

H LEHMANN

University of Cambridge, UK.

Health care in India

SIR,—Anil Agarwal's article on 'barefoot doctors' in India (30 August, page 716) creates the impression that a new health policy was launched in April 1977 after the Janata Government took over. In fact, the community health worker (CHW) made his appearance in the planning of the Ministry of Health of the government of India much earlier. In 1972, he was called the Rural Medical Practioner (RMP). The previous government had hoped to deliver a modicum of health care to the entire country, using RMPs, by 1976. It is apparent that the goal was never reached.

The problem in India at present is not that of an inadequate supply of doctors; it is that of maldistribution. The reason for this geographical lopsidedness in the distribution of health care personnel is that recommendations of the Bhore and Mudlair committees were never fully implemented. Professional isolation, lack of adequate facilities in the practice of scientific medicine and often a lack of personal security are a result of this apathy on the part of successive governments in India in implementing the recommendations of its own specialised committees.

To answer the question with which the article ends: yes, there are alternatives to the CHW scheme. Building physical facilities for health care in the rural areas (as was recommended more than two decades ago) may take another two decades to accomplish. In the meantime, doctors could function efficiently if organised along the lines of the Indian Administrative Service or the Indian Police Service.

The junior-most officers of these cadres very often live in towns, where all the facilities they are used to are easily available, and commute to their places of work in the different villages. Doctors in an Indian Medical Service could do the same.

The children of these doctors could then attend schools which their parents think are reasonable, thereby eliminating one major reason for their reluctance to work in rural areas. Professional isolation would be done away with at the same time, as they could consult with each other on returning to the town after the day's work. Coverage at night could be provided on a rotational basis, akin to the duty hours spent as house-officers in urban hospitals. All this of course implies government expenditure.

Another alternative is completely outside the government. Such a scheme would draw on the private sector — the mini-international companies which Anil Agarwal has described in another of his articles (23 August, page 625).

It has been shown to work on an experimental basis, as the Social Work and Research Centre, at the village of Tilonia in Rajasthan. Why not use it as a model for the other parts of the country as well? Contrary to Agarwal's conclusion, one would hope that CHW scheme or the RMP scheme, call it what you will, does die a speedy death. If not, such half baked medical care will bring untold misery to a vast and gullible public in rural India.

Yours faithfully,

BEHRAM PASTAKIA,

Michael Reese Hospital and Medical Centre, Chicago, US.

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news and views

Production and respiration in animal communities

from Robert M. May

A WIDELY-quoted generalisation about natural ecosystems is that the efficiency of energy transfer from one trophic level to the next is around 10%; that is, about 10% of the net production of plants ends up as net production of herbivores, about 10% of this makes its way into net production of the first level of carnivores, and so on. The generalisation is largely based on studies of freshwater lakes and of laboratory aquaria, conducted in the 1950s. One early articulation of this notion is in Slobodkin's elegant and influential *Growth and Regulation of Animal Populations* (Holt, Rinehart and Winston; 1961), where his speculative list of candidates for valid ecological generalities leads off with "Food-chain efficiencies and ecological efficiencies in nature are approximately constant for all species".

Unfortunately, subsequent research on terrestrial and on other kinds of aquatic communities has overthrown this appealing generalisation, showing that the efficiency of energy transfer from one trophic level to the next can vary very widely. This is a pity, for valid 'ecological laws' are thin on the ground, and we can ill afford to lose any of the few we thought we had.

To determine overall efficiencies of energy transfer, two questions must be answered. First, what fraction of the net production at one trophic level is actually assimilated by creatures at the next level? Second, how do these creatures apportion the assimilated energy between net production (growth and reproduction) and respiration (maintenance costs)? The second question is amenable to fairly precise answers, but the first question is messier, as it can involve both particularities about the fraction of material that is assimilated rather than excreted by a given species and generalities about the overall fraction of net production at one level that is actually used (consumed) by the next level. Some of these problems and ambiguities can be made more explicit by considering, say, mice and weasels. If we focus on the weasels, it is in principle straightforward to determine the efficiency with which 1 gram or 1 calorie of mouse eaten is transformed into grams or calories of weasel. If we focus on the mouse population, it is hard to determine what fraction of their total biomass appears as net production in the next trophic level. Indeed, the answer ultimately depends on how we keep the books; the very notion of 'trophic level' does not stand up to close examination (where, for example, do the decomposers belong?).

Humphreys (*J. Anim. Ecol.* **48**, 427; 1979) has recently drawn together a large body of literature, to determine the relationship between annual production P and respiration R in natural populations of animals. This synoptic study sheds light on the general issues discussed above. It also has more immediate practical applications. If a relation between P and R can be confidently established for a given group of organisms, then for a species in this group only one of the two quantities need be measured directly, which can be a helpful short-cut in compiling energy budgets for communities; several people have used the earlier study by McNeill and Lawton (*Nature* **225**, 472; 1970) for this purpose. Humphreys analyses a total of 235 energy budgets culled from the literature, and he emphasises that a great variety of different assumptions and possible biases have gone into the individual studies.

Examining regression relations between P and R, Humphreys shows that homeotherms (loosely, warm-blooded animals) can be separated into four significantly different groups: insectivores; birds; small mammal communities; and other mammals. Poikilotherms (cold-blooded animals) separate into three groups: fish and social insects; non-insect invertebrates; and non-social insects. The invertebrate groups further permit significant separation into trophic categories of herbivores, carnivores and detritus feeders. In no case is the relation between P and R significantly different from a simple linear one. The fruits of Humphreys' analysis are summarised in Table 1, which shows the mean 'production efficiency' $P/(P+R)$ or fraction of assimilated energy ($A = P+R$) that is devoted to net production, for the various groupings.

Several interesting patterns emerge. Both for non-insect invertebrates and insects other than social insects, the production efficiency is significantly lower for herbivores than for carnivores and

Table 1 Mean production efficiency, $P/(P+R)$, for various groups of animals.

Group	Mean production efficiency (per cent)	Sample size
Insectivores	0.9	6
Birds	1.3	9
Small mammal communities	1.5	8
Other mammals	3.1	56
Fish and social insects	10	22
Non-insect invertebrates	25	73
Non-social insects	41	61
Non-insect invertebrates		
herbivores	21	15
carnivores	28	11
detritivores	36	23
Non-social insects		
herbivores	39	49
detritivores	47	6
carnivores	56	5

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Table 2. The assimilation efficiencies, or percentage of plant production consumed by feeding-animal species, for various systems.

Plant	Consumers	Percentage of productivity consumed
Beech trees	Invertebrates	8.0
Oak trees	Invertebrates	10.6
Maple–beech trees	Invertebrates	6.6
Maple–beech trees	Invertebrates	5.9
Tulip–poplar trees	Invertebrates	5.6
Grass + forbs	Invertebrates	4-20
Grass + forbs	Invertebrates	<0.5
Alfalfa	Invertebrates	2.5
Sericea lespedeza	Invertebrates	1.0
Grass	Invertebrates	9.6
Aquatic plants	Bivalves	11.0
Aquatic plants	Herbivorous animals	18.9
Algae	Zooplankton	25.0
Phytoplankton	Zooplankton	40.0
Marsh grass	Invertebrates	7.0
Marsh grass	Invertebrates	4.6
Meadow plants	Invertebrates	14.0
Sedge grass	Invertebrates	8.0

detritus feeders. A plausible explanation is that biochemical conversion efficiencies are higher for animals eating other animals than for animals eating plants. Other patterns are shown by Humphreys to be conspicuous by their absence: there is no significant correlation between production efficiency and the magnitude of production (that is, no correlation between P/R and P or R); there is no correlation between production efficiency and animal weight; and, with the groups set out in Table 1, species with different habitats (aquatic and terrestrial) do not have significantly different production efficiencies. Humphreys makes the further point that "there is no quantum jump in production efficiency between poikilothermic and homeothermic animals", but I think Table 1 suggests such a distinction is real (with homeothermic production efficiency typically in the range 1-3%, poikilothermic in the range 10-40%). Admittedly the scatter around the mean values for a given group is such that some social insect species have production efficiencies lower than some mammal species, so that there is no 'quantum jump' between homeotherms and poikilotherms, but the tendency for the typical poikilotherm to have a production efficiency an order of magnitude larger than that of the typical homeotherm remains. Warm-blooded beasts pay a noticeable cost, relative to cold-blooded ones, in order to keep their metabolic machinery ticking over at a constant temperature.

For a community of interacting species, we can get some idea of the overall 'food-chain efficiency' with which energy flows from one trophic level (n) to the next ($n+1$), by combining the mean production efficiencies at level $n+1(P_{n+1}/A_{n+1})$ with estimates of the fraction of the productivity at level n that actually is consumed (the assimilation efficiency, A_{n+1}/P_n). Pimentel, Levin and Soans (*Ecology* **56**, 381; 1975) have brought together several rough estimates of the percentage of plant production that is consumed by the animal species that feed upon it; their compilation is summarised in Table 2. As mentioned above, any such estimates of assimilation efficiencies suffer, *inter alia*, from the arbitrariness inherent in a crude classification into 'trophic levels'. Convolving Table 1 with Table 2, we see that food-chain efficiencies can vary over two or more orders of magnitude, from less than 0.1% to more than 10%.

In the early 1960s, the tentative '10% rule' engendered enthusiasm for ecological generalisations. The subsequent collapse of the rule, giving way to the complicated variety of patterns shown in Table 1, has, in my opinion, led to an excessive disenchantment with such generalisations. I think the time is ripe to return to these questions, trying to understand the patterns documented by Humphreys and others, both from 'below' (in terms of thermodynamic constraints on production efficiency in different kinds of animals) and from 'above' (in terms, for example, of the possible constraints that energy flow may impose on food web structure). □

Order in amorphous polymers

from Paul Calvert

THE extent of order in the amorphous state of polymers has been debated for many years. Likening the polymer melocules to strands of spaghetti it is difficult to see how they can be packed to high densities unless the strands are largely arranged parallel to their neighbours. After the Chemical Society's recent Faraday Discussion* on Organisation of Macromolecules in the Condensed Phase the situation seems much clearer than two years ago (see *News and Views*, **271**, 507; 1978).

Two types of order have been postulated for amorphous and glassy polymers, the orientational correlations just mentioned and local density fluctuations with regions of tight packing separated by a less dense matrix or by boundary zones. The latter were apparently demonstrated by Yeh and Geil about 10 years ago. They observed 2.5 nm nodular structures in many glassy polymers with electron microscopy in bright and dark field as well as in fracture surface replicas. At the recent meeting D.R. Uhlmann (Massachusetts Institute of Technology) reviewed his small angle X-ray scattering (SAXS) results which show only enough scattering to be consistent with the small thermal fluctuations in density, frozen in at the glass transition temperature in polymethylmethacrylate, polyethylene terephthalate, polycarbonate, polyvinyl chloride and polystyrene (PMMA, PET, PC, PVC, PS). This does not eliminate the possibility of heterogeneities whose density differs significantly from the bulk density but they must be present in very small quantities. Uhlmann also concluded that the bright and dark field electron microscope observations of small nodules were due to electron diffraction effects.

Thus the 'typical' amorphous polymers are essentially homogeneous. Epoxy resins are not but SAXS cannot distinguish between small quantities of voids and larger quantities of low or high density nodules. G.C. Stevens (CEGB, Leatherhead) said that small aggregates could be detected in unreacted liquid epoxy resins by light scattering. P.H. Geil (Case Western Reserve University) pointed out that he had seen annealing of nodular structures in amorphous polyethylene (PE) and in plasticised and unplasticised PVC. It does seem reasonable that crystallisable polymers such as PE should form crystalline nodules at low temperatures. PVC is frequently partly crystalline and its structure is most dependent on the polymerisation conditions, so any strange behaviour is plausible, and important, in this polymer.

Thus there are heterogeneities in some systems and possibly in small amounts in

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'typical' polymer glasses, which could still affect the fracture and optical properties. The nodules often seen on fracture surfaces seem to be an artefact.

Most of the evidence presented at the discussion was against orientational order but most of it was on PE and paraffins which are atypical in that the chains are very flexible. E.W. Fischer (University of Mainz) and P.J. Flory (Stanford University) reviewed a range of techniques which point to a lack of regular order. Small angle neutron scattering shows that chains have the same radius of gyration in the glass and melt as for the ideal random walk found in θ-solvent. This result seems quite general, having now been seen for PS, PMMA, PE, polypropylene and polyoxymethylene. Orientational order would be expected to lead to chain expansion, whereas if anything this random coil is a rather contracted state. Infrared and Raman results show that probabilities of *trans* and *gauche* conformations are similar in the glassy, liquid and solution states.

Magnetic birefringence and depolarised light scattering, new techniques in this context, both suggest that chains are not parallel for more than about three carbon atoms as opposed to the strong orientation seen in liquid crystals. According to Fischer polystyrene is similar to polyethylene in this respect. A.H. Windle (University of Cambridge) described the impossibility of fitting wide angle X-ray scattering data using models with straight, parallel polyethylene chains where random arrays of straight chains of four or five carbons worked quite well. On the other hand for polytetrafluoroethylene, a very stiff chain polymer, bundle models worked better. These results used scattering beyond 3 $(Å)^{-1}$ which are mostly attributable to interactions of neighbouring groups within chains and so are not really a reliable guide to packing between chains.

The weakness of the available data was demonstrated, perhaps unintentionally, by W. Pechold (University of Ulm). His meander model, in which bundles of chains fold into cubic arrays, came in for much criticism and is unrealistic, but does fit most of the available data. Also no alternatives were presented in such detail; most workers prefer to avoid committing themselves on the exact nature of the packing. Thus the chain is random in terms of its radius of gyration and the local bends and curves are the same in glasses as in solution. As for how they actually fit together we can either look more seriously at spaghetti or ignore the question as unimportant. □

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Replication dissected

from Barry Holland

AT a recent meeting on the control of replication of bacterial chromosomes and plasmids* a mass of genetic evidence was presented that initiation of DNA replication of several plasmids is negatively controlled — that is, through removal of repressor. Even in the case of the high copy number plasmid R6K, where it is known that a plasmid-determined protein (π) is required for initiation both *in vivo* and *in vitro* it was argued that synthesis of this protein is also under autoregulatory negative control (R. Kolter, University of San Diego).

Although the genetic evidence for the repressor is now overwhelming its nature remains elusive. Several participants reported that a DNA fragment coding for a *trans*-acting control substance has been cloned from a number of plasmids but *in vitro* protein synthesis has not yet identified the regulatory substance. This may simply reflect the very small amounts of repressor synthesised, due in part to tight autoregulatory control.

In the past there has been some question of whether plasmid size is a primary determinant of copy number, as plasmids with high copy numbers tend to be smaller. However, there was agreement at the meeting that, providing the primary control of initiation is intact, increasing the molecular size of the plasmid does not in itself affect copy number; however, if plasmid copy number rises substantially in so-called 'copy mutants' copy number is inversely proportional to size and this indicates that total plasmid DNA then becomes the limiting factor (B. Polisky, Indiana University). Discussion of the multiplicity of potential and active origins found in naturally occurring replicons (for example in *B. subtilis*, F and R6K plasmids) exposed a difference in emphasis between participants concerned with understanding what Nördstrom called the 'basic replicon' (the smallest fragment of self-replicating DNA which maintains the copy control of the wild-type replicon), and others, concerned more with the identification of specific sequences which identify regions of chromosomes capable of interacting with replication complexes to promote initiation. As a step towards defining such regions on the *E. coli* chromosome, W. Messer (Max-Planck-Institut, Berlin) has found a 100 base pair fragment of the origin region (*ori*-C) which when integrated into plasmid ColE1 will drive its replication in mutants in which ColE1 replication does not normally occur. On the other hand, Y. Hirota (National Institute of Genetics, Mishima, Japan) reported that a 245-base-pair sequence covering *ori*-C, was the minimum sequence sufficient to rescue ColE1 in similar conditions. Hirota also announced that alteration of even a single base by directed mutagenesis was sufficient to abolish biological activity of the sequence and further studies of this kind should greatly illuminate structure and functional relationships in the *ori*-region.

The presumed significance of the secondary structure identified in all *ori*-regions so far cloned was constantly reiterated and D. Smith (University of California, San Diego) presented new data showing the impressive conservation of possible secondary structures but not necessarily of precise base sequence when the origin regions of *E. coli* and *S. typhimurium* are compared. Overall comparison revealed 88% of conserved bases, and one contributory factor suggested to explain conservation was the presence of 17 GATC sequences, the site of methylation by the *dam* gene, which could therefore be particularly prone to mismatch repair of any incorrect bases in newly synthesised daughter strands.

Some controversy remains over the precise role of the *dnaA* product in the temporal sequence of initiation in *E. coli*. T. Atlung (Institute of Microbiology, Copenhagen) reviewed evidence that the *dnaA* gene product (now convincingly demonstrated to have a subunit molecular weight of 54,000) is an autoregulated, rho-like protein which controls the frequency of initiation, at the primary level, by modulating the rate of transcription in the *ori*-C region. E. Orr (University of Leicester) agreed that the *dnaA* gene product affects transcription but presented evidence in favour of its action at a later step in initiation, subsequent to the primary control step operating in wild-type cells.

Concerning the role of a specific RNA primer at an early stage in initiation, Messer reported that the so-called 'ori-RNA' described previously in his laboratory, does not hybridise with DNA from or near the origin region. Despite this setback there was still general agreement that transcription of a specific ori-RNA primer and/or transcriptional activation to open up the *ori* region, must be an essential early step in initiation. Perhaps indicating the need to unwind the *ori* region, several reports suggested an important role for the enzyme DNA gyrase in initiation. Orr *et al.* and N. Fairweather *et al.* (University of Leicester) described a thermosensitive gyrase mutation mapping in the *cou* (*gyrβ*) gene which prevents colony formation. Nuclear organisation, initiation of DNA replication and placement of septa are all apparently defective in the mutant although, intriguingly, the rate of DNA chain elongation is not significantly affected,

*The 2nd EMBO Conference on the control of replication and the partition of bacterial chromosomes and plasmids was held on 20-24 September in Leicester and organised by Kurt Nördstrom and Bob Pritchard.

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thereby raising doubts about previous assumptions that this enzyme is essential for replication fork movement.

The long haul towards the characterisation of specific components involved in the association of *ori*-C with the cell envelope took an important step forward with the identification of a 65,000 molecular weight membrane protein by A. Jacq *et al.* (University of Paris) and H. Lother *et al.* (Max-Planck-Institut, Berlin) which binds to at least two specific single-stranded DNA sites on the cloned *ori*-C region. Although the precise localisation of this protein in the envelope remains to be established, other findings (H. Wolf-Watz, Umea University; M. Schaechter, Tufts University) that different outer membrane proteins are apparently present in isolated origin-membrane complexes continues to complicate ideas on the actual organisation of such complexes. N. Sueoka (University of Colorado) reported that mutations in a *B. subtilis* gene *dnaB1* (coding for a 35,000 molecular weight membrane protein) block initiation at high temperature, an event which seems to coincide with the loss of chromosomal origins (or plasmids formed from cloned origins) from the membrane fraction both *in vivo* and *in vitro*.

The problem of the relationship between the phenomena of incompatibility of related plasmids and the replication functions of such plasmids was clarified considerably by the general agreement that incompatibility is due to the existence of a *trans*-acting mechanism controlling copy number which fixes the average number of plasmid copies per cell. However, although incompatibility based on competition for a few membrane sites as suggested originally by Jacob, Brenner and Cuzin, was not generally accepted, most participants were still reluctant to concede that random assortment of plasmid molecules at division is sufficiently precise to explain the very low frequencies of plasmid-free clones which are observed even with low copy number plasmids. Several segregation or partition mechanisms were therefore proposed. In particular, the suggestion (T. Hashimoto-Gotoh, Max-Planck-Institut, Berlin) that one pair of plasmid molecules was attached to the cell membrane and partitioned accurately whereas other copies partitioned at random seemed attractive to many.

K. Nördstrom (Odense University) described *par* mutants of plasmid R1 in which copy control is maintained but a few per cent of plasmid-free clones arise in each generation. The wild type *par* function was cloned separately from *rep* functions and since the fragment failed to complement *par*⁻ mutants in *trans*, *par* may be a *cis*-acting DNA sequence essential for membrane binding and hence partition. The novel suggestion by N. Sternberg and M. Yarmolinsky (Frederick Cancer Research Center) that site-specific recombination might also play an important part in plasmid stability or partitioning was received with considerable interest. On the basis of their studies with phage P1, Sternberg and Yarmolinsky proposed that a site-specific recombinational hot-spot ensures that plasmid dimers or higher multimers created through the strong pressures for homologous recombination are likely to be separated and therefore efficiently partitioned. In support of this C. Barton (University of Wisconsin) reported the apparent impossibility of isolating dimers of the large plasmid NR1 *in vivo*.

On a related theme J. Broome-Smith (University of Leicester) described the site-specific, *recA* independent, recombination

How to make a drumlin

from Ian Smalley

THOSE streamlined whale-back hills of glacial till (boulder clay) first recognised and named by M.H. Close in Ireland in 1867 still present problems. Drumlins have fascinated geomorphologists perhaps more than any other glacial landform — or so claim Whittecar and Mickelson (*J. Glaciol.* **22**, 357; 1979) — their symmetry, spatial groupings, and the variability of their lengths, heights and materials have led to a variety of theories of drumlin formation. In general they are believed to have been formed by the moulding action of the ice-sheet on newly-deposited moraine.

Whittecar and Mickelson have investigated the Waukesha drumlin field which contains about 650 drumlins and related streamlined features surrounding Waukesha, Wisconsin, about 20 km west of Milwaukee. The field was formed by ice advancing out of the Lake Michigan basin approximately 14,500 years ago and it abuts the Kettle interlobate moraine to the west and is overlapped by a younger set of moraines to the east.

On the basis of the internal structures of the drumlins Whittecar and Mickelson propose a sequence of five events involved in their formation. The particular structures may be unique results formed by special situations in ice temperature regimes, groundwater flow, stratigraphy, and sediment bulk densities. Whatever the features shown, however, they often indicate that material was being moved into the drumlin from below. A growth relationship may exist that is based on the rates of material moving into the drumlin from below and rates of erosion of the drumlin form.

J. Menzies of Brock University, Ontario has also proposed a new theory of origin (*Earth Sci. Rev.* **14**, 315; 1979). He suggests that the new proposal might point the way to some fruitful field research and have a unifying effect on the diverse disciplines involved in the drumlin problem. He notes that it is commonly observed that subglacial debris emerging into the proglacial zone from beneath an ice mass is slurry-like, having a very high water content. Such material would not be capable of developing into a drumlin-like obstruction at the base of an active ice mass since it tends to be rapidly removed due to its extremely low shear strength (the result of a high pore water content) in relation to the basal shear stresses of the ice.

For such material to survive at the ice/glacier bed interface a marked change in its shear strength must occur, and the most likely process to bring about such a change is pore water dissipation. With stresses applied to the material at the interface the pore water will, if routes are available, dissipate away from points of high pressure. The rate of dissipation can be expected to vary depending on the lengths of the routes and on the size of the voids and thus on the texture and consolidation characteristics of the materials. If the water can escape from the interfacial material a relatively rigid structure might be formed which could become a drumlin. Thus the essence of the Menzies theory is that drumlin formation is controlled by variation in pore water pressure in the glacial till material.

The pore water theory bears some similarities to the dilatancy theory of drumlin formation (dilatancy is the expansion of closely packed granular masses with change in shape — in this case under ice pressure) but invokes a different mechanism to provide critical stress levels within the drumlin material. According to Menzies the dilatancy theory still holds a certain sway; he divides drumlin theorists into those who concur completely with dilatancy theory and those who do not reject the theory but who have alternative hypotheses. Crozier (*Can. Geogr.* **14**, 181; 1975), studying the Peterborough drumlin field in Canada, devised a test for the dilatancy theory and claimed convincing support for it.

In a second paper (*J. Glaciol.* **22**, 373; 1979) Menzies gives more details of his formation mechanism. Two mechanisms of pore-water removal are possible. First, pore water may be removed from localised patches within a mobile layer of till at the ice/glacier bed interface, thus creating nuclei of higher-strength till around which deforming till may adhere. Second, removal of water, initially from the thin water film at the base of a glacier, may result in increased pressure melting of the ice leading to till melt-out and subsequent loss of pore water from the deposited till. Menzies rather favours the first mechanism. □

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of ColE1 (and ColK) with a comobilisable plasmid during transfer. It was suggested that such recombination is a consequence of the normal mobilisation process, involving strand cutting at, and rejoining of, the similar transfer origins. M. Masters (Edinburgh University) also described the *recA* independent integration of plasmids bearing *ori*-C into a specific site near the origin of the bacterial chromosome and suggested that this event might also involve a site-specific endonuclease, perhaps involved in this case in initiation of chromosomal replication. Finally, R.H. Pritchard (University of Leicester) recalled the puzzling findings, discussed at the previous workshop, that several high copy number 'synthetic' plasmids (for example, λdv,) and copy mutants of Clo DF13 were extremely unstable in *recA*$^+$ but less so in *recA*$^-$ hosts. Many participants therefore left the meeting intending to search for site-specific recombination mechanisms, perhaps hitherto recognised as *par* functions, evolved by plasmids either to reduce recombination between plasmid molecules or to resolve multimers when they occur. □

Transposable elements and chromosomal rearrangements in cancer — a possible link

from Ruth Sager

THE existence of transposable genetic elements which can insert into different chromosomal sites and thereby modulate gene expression was first described in maize by Barbara McClintock more than 25 years ago. More recently, similar elements — insertion sequences, transposons, and the mutation-inducing phage mu — have been found in bacteria; the molecular basis of transposition is under investigation in bacterial systems and in maize. The likelihood that the non-random chromosome changes associated with particular kinds of cancer might also be mediated by transposition events was the subject of a recent workshop entitled 'Chromosomal Transpositions as Causal Agents in Cancer'.*

Chromosome changes

Consistent chromosome changes affecting specific chromosome segments have now been revealed in banding studies of chromosomes from thousands of patients with a variety of haematological malignancies, and similar associations of chromosome aberrations with other kinds of cancer are being found with increasing frequency. J. Rowley (University of Chicago) described the chromosome gains, losses, deletions, and translocations associated with leukaemias, especially chronic myeloid and acute nonlymphocytic leukaemias; these chromosome changes arise in bone marrow cells. In the inherited form of retinoblastoma, however, patients have an abnormality of chromosome 13 in every cell. A. Mark (Central Hospital, Falk, Sweden) reported that chromosome 22 is regularly lost in human meningiomas, and that many 'double minute' chromosome fragments are present in astrocytic gliomas. In non-malignant lymphomas, short segments from other chromosomes are inserted into a particular region of the long arm of chromosome 14 (14q+); and trisomy for chromosome 3 is also found. A similar 14q+ chromosome was also seen in B cell acute lymphocytic leukaemia.

G. Klein (Karolinska Institute, Stockholm) reported that the 14q+ chromosome seen in Burkitt's lymphoma is a reciprocal translocation with chromosome 8, and is found in tumour cells but not in the peripheral circulation. In recent studies, constant break points have been identified in both translocated chromosomes 8 and 14.

Klein proposed that the 8;14 translocation results from random changes followed by cell selection occurring in stem cells made hyperplastic by infection with Epstein-Barr virus and by additional mitogenic stimuli such as malaria. He stressed the importance of host chromosome changes in the development of virus-associated cancer. Rowley proposed that the evident specificity of break points and rearrangements in cancer-associated chromosome aberrations might result from preferential pairing of homologous DNA regions or from random rearrangements giving rise to cells with a proliferative advantage. Transposable genetic elements like those in maize have the properties that could determine the rearrangements seen in cancer progression.

Assays for tumorigenicity

Interpreting the phenotypic expression of particular chromosome changes requires cellular assays of the malignant phenotype. A. Pardee (Sidney Farber Cancer Institute, Boston) compared growth control in normal and tumour cells transformed by DNA viruses, RNA viruses and chemicals. In normal cells, growth is controlled externally by cell-cell interactions and by growth factors (proteins and small peptides) supplied by the vascular system. Tumour cells no longer respond completely to external growth controls; considerable variation is seen from one tumour type to another in the particular responses that have been lost. Pardee proposed a unified theory of growth control in which a key protein, the 'restriction protein', regulates an on-off switch that permits or blocks entry of cells into a new round of the cell cycle. He further proposed that the rates of synthesis and degradation of this protein vary such that tumour cells generally have a higher level than normal cells but variation also exists among tumour types.

J. Ponten (Uppsala) proposed three requirements for normal cell growth: low molecular weight components; growth factors; and the solid substrate. In the presence of one of Ham's recent new media for human cells, the growth factor most clearly required by glial cells is PDGF, the platelet-derived growth factor. With respect to substrate attachment, Ponten has used circular palladium islands of controlled size to which cells attach to show that normal glial cells require a minimum of 500 square micrometres for growth regardless of growth factor availability. This result is consistent with Folkman's evidence that cell shape is of controlling importance in growth, but does not distinguish between shape and anchorage.

Ponten stressed that with glioma cell lines established from human tumours, no single phenotypic trait correlated well with tumorigenicity *per se*. He proposed that population immortality is the only reliable qualitative distinction between normal and tumour cells.

Additional cellular changes leading to metastatic potential were discussed by M. Burger (Biozentrum, Basel) who proposed that specific surface carbohydrate changes may influence the degree to which some tumour cells metastasise. Starting with the B16 mouse melanoma cell line, he used resistance to wheat germ agglutinin as a selective system to recover mutants with altered surface glycoproteins. Some variants that 'home' preferentially to the liver were also isolated for analysis on the basis of organ selectivity.

In summary, loss of growth control and immortality were taken as the best cellular evidences of transformation, and further changes were considered necessary for establishment of tumorigenicity. The relation of cell shape and anchorage to tumorigenicity remained unclear.

Chromosomal changes and malignant phenotype

Genetic analysis of cells in culture provides a means to link chromosomal changes with specific phenotypic alterations involved in malignancy. R. Sager (Sidney Farber Cancer Institute, Boston) described a new Chinese hamster cell line which is a stable diploid (22 normal

*Held in Sils Maria, Switzerland on 8-12 June, 1979 and sponsored by the International Union against Cancer and the Swiss National League against Cancer.

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chromosomes) and non-tumorigenic. Transformed and tumorigenic mutants have been recovered and genetic differences between normal and mutant cell lines have been examined by cell fusion and subsequent chromosome loss from the tetraploid back to the diploid level, during which reassortment of parental chromosomes occurs. Evidence has been found for suppression in cell hybrids of the anchorage requirement and of tumorigenicity, confirming and extending the original studies of Klein and Harris. In some reduced hybrids with 22 chromosomes, anchorage independence and tumorigenicity were re-expressed independently indicating that these traits are under separate genetic control. In addition, fusions of transformed cells with enucleated normal cells to form cybrids have shown that cytoplasmic genes are involved in tumorigenicity. The importance of examining early stages in tumorigenicity in order to identify the initial key changes that occur was stressed.

The methodology of cell hybridisation has been developed and applied by B. Ephrussi and his students to the analysis of differentiation. M. Weiss (CNRS, Gif-sur Yvette) described the extinction of sets of phenotypic traits of rat hepatoma cells following fusion with normal or malignant mouse cells, and the subsequent re-expression of individual phenotypes coincident with loss of various chromosomes. For example, when rat hepatoma cells that produce albumin are fused with pigmented mouse melanoma cells that don't, the resulting hybrid clones are unpigmented and do not produce albumin, but they give rise to hybrid subclones that have lost chromosomes and segregate for pigmentation and albumin production. Stable mutants have been recovered from the hepatoma line that have lost some or all of their differentiated liver functions; and revertants from these mutants have regained the full set of enzymes. The reversion process is not increased by X-ray or chemical mutagens.

The extinction of sets of phenotypes in cell hybrids followed by full or partial regaining of activity, in parallel with chromosome elimination, strongly resembles suppression of tumorigenicity and its reappearance in reduced hybrids. Both systems can be interpreted in terms of regulatory signals that can turn on or turn off expression of sets of genes. As with tumorigenicity, the genetic basis of extinction and re-expression needs to be examined at the DNA level.

The relation between cancer and normal cell differentiation was stressed by E. Gateff (University of Freiburg) who described twelve unlinked *Drosophila* mutations which are larval lethals. The mutant genes interfere with development of specific tissues or cell types in a stage-specific way and thus are developmental mutations affecting rates of cell proliferation and morphogenetic processes. In addition, neoplastic sublines have been derived from wild-type imaginal disks maintained in prolonged subculture *in vivo*. One of these lines has shown a capacity for reversal to the differentiated state, suggesting the regulation by genetic on–off switch. The wealth of descriptive detail that Gateff has produced over the past few years makes this material a valuable resource and a challenge for anyone who can develop a molecular approach to its analysis.

The possibility that the cell cycle might be regulated by one of the enzymes involved in initiation of DNA replication was raised by B. Alberts (University of California, San Francisco). He suggested that eukaryotic DNA replication might profitably be approached by looking for eukaryotic enzymes with functions analogous to the prokaryotic ones. Alberts also discussed the possibility that gene regulation might occur in some instances at the supra-DNA level of chromosome organisation.

Transposition and carcinogenesis

Of central interest to the workshop were molecular aspects of gene organisation such as the eukaryotic split genes described by P. Chambon (Strasbourg) and mechanisms of transposition in bacteria described by N. Kleckner (Harvard University, Cambridge), A. Toussaint (Brussels), and H. Saedler (Freiburg), subjects previously reviewed in *Nature*.

Despite the differences between prokaryotes and eukaryotes in gene and in chromosome organisation, unanticipated similarities have been discovered with respect to the existence and roles of transposable genomic fragments.

Saedler connected the prokaryotic and eukaryotic systems by a brief account of the discovery of chromosome transpositions in the corn plant *Zea mays* by Barbara McClintock (who unfortunately was unable to attend). McClintock discovered the existence of gene-control elements capable of inducing chromosome breaks and rearrangements and of modulating gene expression both qualitatively and quantitatively. She characterised several classes of these elements and showed that their transposition from one chromosomal location to another can be stimulated by chromosomal breakage. Whether transposition is a regular feature of their function in normal development is not known, but the transposition process is clearly under genetic control, since its time and frequency of occurrence is highly non-random and is inherited as a property of the controlling element.

The similarities in behaviour of the controlling elements in maize and the more recently discovered insertion elements and transposons of bacteria was a principal factor in the design of this workshop. In McClintock's words: "Should a genome-disturbing event appear, such as by a cancer-inducing agent, the result could be induction of transposition of elements that can serve to control gene action. The insertion of such elements at given gene loci could initiate those genomic changes that lead to a cancerous state of the cell."

According to this view, initiation of tumorigenicity by a chemical or a virus might result from a subtle chromosome change which would precipitate subsequent chromosomal instability. Progressive development of malignancy might then depend on random karyotypic reorganisation and selection of new cell types that have lost growth control and gained other traits that facilitate growth in the given environment.

This approach to carcinogenesis may be particularly fruitful in suggesting new kinds of experiments in which the process of tumorigenesis is analysed in terms of transposition and chromosome rearrangement. Understanding the underlying karyotypic alterations that occur during tumour cell selection may provide new insights into the choice of effective anti-cancer drugs, both clinical and prophylactic. □

Pollen problems

from Peter D. Moore

WHEN interpreting the fossil pollen content of past lake sediments in terms of the contemporaneous, pollen-producing vegetation, one needs to consider the spatial origins of the pollen assemblage. Some will have arrived from the atmosphere directly, either blown from nearby plants, or carried down by drops of rainwater which effectively 'forage' for particulate matter in the atmosphere (McDonald *Science* **135**, 435; 1962). Other pollen will have been transported into the lake by the input streams.

Stream-borne pollen may itself be derived from a variety of sources. Some may have been washed from leaf and other surfaces in the catchment, where it had become impacted from the atmosphere (Chamberlain & Chadwick *Ann. appl. Biol.* **71**, 141; 1972). Some may be derived from other types of deposit, such as peat or undecomposed humus and litter from soils in the catchment. This latter source is of particular concern to the palynologist, for such pollen could be considerably older than the remainder of the pollen load in the stream and would distort the general pollen record within the lake basin. Some attempts have been made to separate this inwashed pollen component within fossil

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pollen assemblages (for example Birks *New Phytol.* **69**, 807; 1970) on the basis of the different types of damage which such grains will have suffered during transport, but generally little is known of its importance.

An experimental approach to this question has been conducted by Bonny (*J. Ecol.* **66**, 385; 1978) making use of the large tubes inserted into Blelham Tarn in the English Lake District by Lund (*Mitt. Int. Verein. Limnol.* **18**, 71; 1972). These tubes are 45 m in diameter and extend from the surface to the bottom of the lake (11-12 m) and thus effectively isolate a body of water from the remainder of the lake. Bonny established pollen traps at the lake surface and underwater, both inside and outside the tubes; traps inside the tube would be expected to receive the atmospheric pollen component, but to lack the pollen transported into the lake by streams. She found that the annual catch of pollen within the tubes was only 15% of that caught outside. This suggests that as much as 85% of the pollen submerged in the lake may have been brought there by stream transport, though this figure may be inflated by the input from marginal, eroding banks and organic deposits.

Pennington (*New Phytol.* **83**, 189; 1979) has now approached the problem from a different angle, by comparing the pollen record within the sediments of an enclosed basin lake with no inflow streams with one into which streams flow. Blelham Tarn was again taken as an example of the latter, and Whinfell Tarn, a 5.5 hectare basin also in southern Cumbria, as an example of the former. Sediment cores were extracted and the pollen stratigraphy determined using absolute techniques, covering about the past 7,000 years.

The general course of vegetation history as recorded by the pollen was similar in the two lakes. There were fewer irregularities in the enclosed basin, perhaps as a result of the smaller influence such processes as local forest clearance would have had upon erosion and pollen input. On examining absolute pollen influx into the two lakes over the past 7,000 years, however, considerable differences were apparent. Current pollen sedimentation in the surface muds of Blelham Tarn are about nine times greater than those at Whinfell Tarn. Differences between the two basins, however, have not always been so great. About 5,000 years ago, before the local deforestation of the two sites, the stream-fed lake received only twice the pollen influx of the closed basin. Following catchment deforestation this rose to about four times and the difference has been rising rather erratically ever since. This seems to be due to the erosion of organic, mor humus from the cleared forests of the Blelham catchment and the stream-borne input of this older pollen into the lakes, raising the pollen influx values by about five times. This effect is not seen in the enclosed basin. The subsequent erratic behaviour of the influx curves at Blelham Tarn can be correlated with further soil disturbance by such processes as ploughing and cultivation.

The conclusion, therefore, is that the stream contribution to a lake's pollen content will vary with the vegetation and soils of the catchment. Where this consists of stable forest, stream-borne pollen may be less than 50% of the total pollen influx. Forest clearance or soil cultivation releases any acidic organic matter into streams and may inflate this source of pollen to about 85% of the total input.

Such contamination with old pollen is a serious disadvantage when trying to interpret pollen data and indicates that a closed basin provides a more reliable pollen record than an open one. The alternative, of course, is to choose an ombrotrophic (rain-fed) peat site in which all pollen input is atmospheric in origin. □

Chemical recognition in biology

from James Ofengand

SPECIFICITY of reaction is the hallmark of all biological processes. In order to achieve specificity, biological molecules have learned how to recognise one another. Understanding the chemical basis for recognition has been the goal of biochemists and molecular biologists for many years, and none has been more persistent in its pursuit than Fritz Lipmann, whose 80th birthday recently was marked by an EMBO Workshop on Concepts of Chemical Recognition*. Many of the participants were former associates of Professor Lipmann, and while their interests have diverged widely over the years, the approaches taken still reflect in a characteristic way their common exposure to the view that the behaviour of biomolecules can ultimately be explained in terms of the laws of physics and chemistry.

The meeting opened with a series of talks on protein–ligand interactions. C. Walsh (Massachusetts Institute of Technology) discussed suicide substrates, structural analogues of a substrate which carry a latent reactive group capable of being exposed only by the catalytic action of the enzyme. Since these compounds are only unmasked and activated when at their target enzyme, they are well suited for *in vivo* studies as well as for consideration as chemotherapeutic agents. An example of the latter possibility is clavulanic acid (Fisher *et al. Biochemistry* **17**, 2180, 2185; 1978). This is a penicillin analogue which inactivates the β-lactamase responsible for most of the penicillin resistance encountered in pathogenic organisms. Walsh also pointed out the importance, *in vivo*, of a low partition ratio. This is the ratio of activated product which diffuses away from the active site to that which reacts with the enzyme and inactivates it. One substrate cited whose partition ratio approaches zero is the naturally occurring cyclohexadiene amino acid, gabaculine, which inactivates various GABA transaminases. The development of suicide substrates for pharmacologically important enzymes should be a rational approach to chemotherapy.

M. Perutz (MRC Laboratory of Molecular Biology, Cambridge) drawing primarily on X-ray crystallographic data, described how haem proteins recognise their substrates. Haemoglobin and myoglobin prefer O_2 over CO because of steric interference of the side chain of HisE7 which blocks linear addition of the CO molecule but not the angular addition of O_2. This conclusion was verified using Hb Zurich, in which HisE7 is replaced by Arg. Similarly, cytochrome b_5 is an electron carrier rather than an O_2 carrier in this case because HisE7 is only 2 Å from Fe^{2+} instead of the 4 Å in haemoglobin or myoglobin so that even O_2 is sterically excluded.

Protein–protein recognition was discussed by M. Kamen (University of California, San Diego), using the recognition of cytochrome *c* by cytochrome oxidase as the paradigm. Kamen concluded that a pattern of positive surface charges produced by exposed lysine residues on one face of the cytochrome *c* molecule determines the nature of the interaction with the oxidase. Although the overall net charge on both proteins is negative, the concentration of positive charges on one surface allows electrostatic forces to pull the two molecules together, and the specific pattern of these charges determines the strength of the interaction and hence the specificity.

The complex bicyclic system for the regulation of glutamine synthetase (GS) activity was discussed by E. Stadtman (National Institutes of Health). This 12-subunit enzyme exists in an adenylated (inactive) and non-adenylated (active) form with each subunit able to accept one AMP residue. The interconversion requires ATP and adenyl transferase (AT), which can both adenylate GS with release of PP_i, and de-adenylate by phosphorolysis to yield ADP. Which of the two activities is catalysed by AT is determined by yet another protein, PII. PII is required for adenylation in addition to AT but does not induce de-adenylation. The latter reaction requires AT, P_i, and the uridylated form of PII, PII-$(UMP)_4$. The interconversion of PII and PII-$(UMP)_4$ is accomplished by a fourth protein, uridyl transferase, which when stimulated by α-ketoglutarate, adds

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the UMP group of UTP to PII, and when glutamine is present hydrolyses PII-$(UMP)_4$ to PII. The net consequence of this elaborate system is that the α-ketoglutarate/glutamine ratio determines the activity of glutamine synthetase, although as Stadtman was careful to point out, the complexity of the system allows more than 40 different metabolites to modify the overall GS activity level. When GS is partially adenylated, 12mers with varying degrees of adenylation and varying specific activities are produced rather than a mixture of fully adenylated and nonadenylated 12 mers.

The subject of the Lipmann Lecture, given by Y. Nishizuka (Kobe University, Japan), was protein kinases. After reminding the audience that Lipmann was also a pioneer in the field of protein phosphorylation (Lipmann & Levene *J. biol. Chem.* **98**, 109; 1932; Lipmann *Biochem. Zeit.* **262**, 3, 9; 1933), Nishizuka described a new class of multifunctional protein kinases discovered in his laboratory. These kinases (molecular weight 77K) are found in various mammalian tissues and require both Ca^{2+} and membranes for activity instead of cyclic AMP or cyclic GMP (Takai *et al. J. biol. Chem.* **254**, 3692; 1979, *J. Biochem.* **86**, 575; 1979). The membrane component can be replaced by certain purified phospholipids or, even more effectively, by the neutral lipid diolein. Ca^{2+} stabilises the enzyme-phospholipid complex as its removal by chelating agents dissociates the protein–lipid complex and destroys kinase activity. Interestingly, dibucaine, a local anaesthetic known to interact with phospholipids, also blocks the activity of this kinase, although it has no effect on cyclic AMP or cyclic GMP-dependent protein kinases. After proteolysis the catalytic fragment (molecular weight 51,000) is fully active without Ca^{2+} and phospholipid. The specificity of the lipid-protein interaction described here should be a fruitful area for further study in addition to the obvious possibilities for transmembrane control implicit in a Ca^{2+} regulated membrane-associated protein kinase.

Protein–nucleic acid recognition was discussed by several speakers. G. Hartman (University of Munich) described some of his group's recent work on the recognition of promoter sequences by RNA polymerase. Although subunits from *M. luteus* and *E. coli*, two taxonomically distant species, are freely interchangeable in reconstituting overall activity, the promoter sequences recognised depend both on the source of enzyme and of the DNA, and vary from no to complete discrimination; analysis of hybrid molecules shows specificity for promoter recognition is due to the sigma subunit.

Recognition of DNA by the protein antibiotic, neocarzinostatin (NCS) was described by I. Goldberg (Harvard University). This antibiotic, which inhibits both bacterial and mammalian cell growth, has been used clinically as an anti-tumour agent. It is a compact protein of 10,700 molecular weight and introduces single strand breaks in DNA at T or A residues probably by splitting the deoxyribose sugar between C_3' and C_4'. A chromophoric group (molecular weight 600) in NCS may be a cofactor.

Specific recognition of satellite DNA by protein was suggested by some recent work from the laboratory of H. Zachau (University of Munich). Each long repeat unit (2,350 base pairs), is made up of four segments, each consisting of variations on the theme of a basic 23 base pair unit which is itself made from a 12 bp and a related 11 bp sequence. Analysis of the complete sequence showed that the extent of divergence as well as the frequency of base substitution at specific positions was characteristic for each segment. A high incidence of symmetry was also found, and provided the basis for Zachau's suggestion that proteins might specifically recognise certain satellite sequences.

Recent experiments on the control of replication of SV40 were described by D. Nathans (Johns Hopkins University) (Shortle & Nathans *J. molec. Biol.* **131**, 801; 1979). Using their localised mutagenesis technique (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2170; 1979) they isolated mutants in the origin region of SV40 DNA, which fell into four classes corresponding to differences in rates and requirements for DNA replication, and were not correctable by complementation with wild-type gene products. Sequencing established the exact nature of the base changes and Nathans proposed that mutants in the *ori* region alter a sequence which is recognised by the T antigen, a viral protein involved in the initiation of viral DNA synthesis. This hypothesis is supported by the isolation of second site revertants some of which were mutants in the T antigen gene.

Various aspects of protein-nucleic acid recognition involved in protein biosynthesis were considered. A. Rich (Massachusetts Institute of Technology) presented some recent experiments (Wrede *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3289; 1979) which revealed unexpected and characteristic differences between the anticodon loop structure of the initiator methionine tRNA, $tRNA_f^{Met}$, and the elongator tRNA, $tRNA_m^{Met}$ in the pattern of cleavage by the single-strand specific endonuclease S1. Both prokaryotic and eukaryotic initiator tRNAs were split at two sites in the anticodon, residues 34 and 35, while elongator tRNAs of prokaryotes and eukaryotes were split at residues 33, 34, 35, and 36 (34–36 are the anticodon). The reason for this characteristic difference in splitting pattern is not known. Rich speculated that the three G-C pairs found in the anticodon stem of all initiator tRNAs, and the only sequence feature common to all initiator tRNAs, might somehow give the anticodon loop a characteristically different structure from that of elongator tRNAs.

H. Wittmann (Max-Planck-Institut, Berlin) summarised the current state of knowledge of the *E. coli* ribosome. The primary sequence of 47 of the 53 ribosomal proteins is now known, and their shape is being studied by a variety of physical methods. A number (S1, S18, S7/12, S21, S5) are very elongated with axial ratios of 7-10:1. Several are modified by acetylation or methylation on side-chains or N-termini and three distinct N-terminal acetylases have been identified, but the purpose behind these elaborate modifications has yet to be determined. The architecture of the ribosomal particle is being studied by chemical cross-linking techniques and by immunoelectron microscopy. Two previously described crosslinks have now been precisely identified, the 8Å link between Lys93 (S8) and Lys166 (S5), and the 0 Å crosslink between Met114 (S7) and Urd1239 (16S RNA). Ultimately, the Berlin group expects to map out most of the contact sites in the ribosome in this way. New immunoelectron microscopic results were the localisation of the 3'-end of the 16S RNA, and of the chloramphenicol binding site at the peptidyl transferase centre.

Y. Kaziro (University of Tokyo) reviewed the way that the energy of hydrolysis of GTP is used to do mechanical work in protein synthesis. Both in A site binding and in translocation, the energy comes from the participation of a protein-GTP complex which induces a change in state such that the reaction proceeds. GTP hydrolysis occurs only subsequent to the mechanical process to release its carrier protein from the ribosome by producing a conformational change in it. Kaziro suggested that this concept of work first, hydrolysis later, might be extended to other biological systems where work is done such as muscle contraction, transport, and other polymerisation reactions. Kaziro has also nearly completed sequencing of the EFTu gene, *tuf*A.

Chemical recognition is being used in cancer chemotherapy to direct antimetabolites to the desired site (N. Kaplan, University of California, San Diego). 6-aminonicotinamide, a potent antimetabolite, has been coupled to polylysine to prevent it crossing the blood–brain barrier, the major cause of its toxic side effects. Polylysine coupling to methotrexate has been used to fool a methotrexate-resistant cell line in which resistance was due to a failure to transport the drug. A third example was the coupling of daunomycin, an inhibitor of DNA and RNA synthesis, to melanotropin in order to concentrate the drug specifically in the melanoma cell. This idea of chemically addressing drugs which normally have no capacity to recognise specific cell types should be a fertile ground for new approaches to chemotherapy. □

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review article

Redshifts and distances

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Evidence concerning the associations between QSOs and galaxies with similar redshifts, and pairs with very different redshifts is reviewed. It is concluded that both kinds of associations are likely to be real.

FOR more than 10 years there have been doubts over whether all redshifts of extragalactic objects are associated with the expansion of the Universe and can be used to indicate distances. The arguments have centred largely on the nature of the redshifts of the QSOs, although Arp[1] has given evidence which suggests that even some galaxies have non-cosmological redshifts, and Tifft[2] has suggested that the redshifts of the galaxies in clusters, in particular, the Coma and Perseus clusters, are correlated with their nuclear magnitudes and has suggested that distances derived by the usual assumptions cannot be valid.

Because there has been no realistic theory which would account for non-cosmological redshifts, the majority feel that there is no good case for this point of view and strenuous efforts are made to disprove the existing evidence. At the same time, some evidence in favour of the cosmological interpretation has been found, and this evidence is regarded more highly.

I have previously attempted to summarise the evidence on both sides of the controversy[3]. Here I will concentrate on findings made largely since then on one aspect of the problem—the apparent associations between QSOs and galaxies—the first assumption being that the redshifts of the galaxies give good measures of their distances.

The investigations fall into several categories:

(1) The programme of looking for QSOs near bright galaxies which has been carried out by Arp and his colleagues.
(2) Searches among specified samples of pairs of QSOs.
(3) Attempts to find associations between QSOs and faint galaxies on the assumption that the redshifts are cosmological.
(4) Studies of the correlations between galaxy samples and QSO samples without any assumption being made as to the nature of the QSO redshifts.

Before summarising the results of these different investigations a few comments should be made. The average QSO redshift z is close to 1 and only a very small fraction of the known QSOs (~5%) have redshifts ≤ 0.2. On the other hand, practically all of the galaxies in the published catalogues are thought to be at distances such that their cosmological redshifts are ≤ 0.2. Thus, except in special cases in which faint galaxies are deliberately sought out and observed, in any associations that are found between QSOs and galaxies, the QSO redshifts will be much greater than that of the galaxies. A further point concerns Arp's samples. The frequent criticism is that while much is made of the peculiar objects, there must be many galaxies whose fields have been searched and in which no QSOs or anomalous redshift galaxies have been found. The implication is that the cases he describes are rare, and thus may be accidental. In fact the limits on observing time and the exacting nature of the photographic work means that this is not true. To carry out such investigations on a large number of fields would take decades at the rate at which dark time is now available on the large telescopes. I believe that the fact that Arp finds so many of these cases suggests that the phenomenon is widespread, and that more cases are not found because no other astronomers are looking.

QSOs near to bright galaxies

Arp[4] has listed 23 cases of QSOs found near bright galaxies. Of these 20 are NGC galaxies, two are IC galaxies and one was not catalogued. The objects are of two types: QSOs which have been found near to bright galaxies such as the 3C QSOs studied many years ago[5], and QSOs which have been found in conjunction with fainter galaxies which are themselves thought by Arp to be associated with bright galaxies with very small redshifts. In such cases the fainter galaxies generally have redshifts much greater than those of the bright galaxies.

Since 1976 several new examples have been found by Arp and his colleagues. A list of all of the systems of galaxies and QSOs which are known has been published elsewhere[6]. Both QSOs associated with galaxies with much smaller redshifts, and fainter galaxies found around QSOs which in some cases have the same redshifts are included.

The list includes 94 large-redshift QSOs close to 55 NGC or IC galaxies with $m_v \leq 13$ and 10 fainter galaxies, several of which are in the Zwicky catalogue. All of these cases involve highly discrepant redshifts. The remainder are nearly all very faint galaxies near to QSOs with $z < 0.45$ chosen by Stockton[7] who was looking for cases in which the redshifts agree. We shall return to them later. Among the pairs with discrepant redshifts, a few have been found accidentally, some by deliberate searches involving a limited sample of bright galaxies and radio QSOs (the 3C QSO galaxy-pairs[5] and the PKS ± 4° QSOs and Zwicky catalogue galaxies[8]) and the majority by looking in the vicinity of galaxies chosen because detailed radio studies indicated some possible peculiarity or because Arp found a peculiar fainter galaxy in the vicinity of the brighter one.

Table 1 shows all of those QSO–galaxy pairs which have separations $\leq 10'$. This table is abstracted from ref. 6. (The separation is either between a bright galaxy and a QSO or between a QSO and a companion galaxy close to a bright galaxy. These latter cases are all due to Arp.) There are 68 QSOs and 54 galaxies in Table 1. Of these, 57 are QSOs associated with NGC or IC galaxies and 11 are QSOs associated with fainter galaxies, five of which are brighter than 15.5 mag.

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Given the heterogeneity of the data, the best way to evaluate the results statistically is to ask, if one looks within a range of angular distances of a chosen set of points, how many QSOs would be expected to be found by chance.

The information that we need to evaluate the result is the surface density of QSOs, and the number of cases (points) that have been examined.

It is generally agreed that the number of radio-quiet QSOs is far larger than the number of radio-emitting QSOs. The surface density of QSOs given by Sandage and Luyten[9] and Setti and Woltjer[10] down to $B = 20$ mag are 10 and 8.5 per square degree. Arp, Sulentic and de Tullio[11] obtain 7 ± 4 per square degree. Savage and Bolton (quoted by Wills[12]) obtain values of 0.2, 1.0 and 3.0 QSOs per square degree to $B < 18.0$, 19.0 and 20.0, respectively. Wills[12] has criticised the problem and has concluded that a value of 20 per degree down to $B = 20.5$ mag is indicated with a very considerable uncertainty. All of the surface densities mentioned above are in good agreement so that we conclude that values of 0.3 QSOs per square degree to 17 mag, 1 QSO per square degree to 18 mag, 3 QSOs per square degree to 19 mag, and 10 QSOs per square degree to 20 mag can be used. At the bright end this number is obviously conservative.

How many fields have been searched? Only Arp has looked extensively, sometimes after a pecularity in the galaxy or companion has been recognised. For example, his study of the vicinity of NGC1073 began after Condon and Dressel[13] reported the discovery of a compact radio source associated with a blue stellar object in the galaxy. The object was found to be a QSO, but it is the only example they found in a study of ~200 galaxies for compact radio sources. Thus this might well be a chance effect. On the other hand the discovery of two more (radio-quiet) QSOs by Arp and Sulentic[14] may be highly significant.

The observing time required to obtain adequate photographic material compared with the limited dark time available for this work leads me to conclude, with Arp, that a maximum of 100 fields have been studied by him and his colleagues. Some of the other objects have been found by accident, and we shall assume at first that the total number of fields that have ever been looked at is 200.

Let $\Gamma(<m)$ be the sky density of QSOs brighter than m. Then the expected number lying within an angular separation θ of arbitrary points is

$$\langle n \rangle = 2.4 \times 10^{-7} N \Gamma(<m) \theta^2$$

where N is the number of tries, Γ is expressed in (arc deg)$^{-2}$ and θ in arc s.

Table 2 gives the number of QSOs expected by chance, as a function of separation from the galaxy and as a function of magnitude of the QSOs, compared with the total number found (N_0). The number that has been found by Arp is designated N_{0A}.

Table 2 shows that the number found within 1, 2 and 3 arc min is far greater than the number expected by chance and thus the result is highly significant.

It may well be argued that the number of tries used to calculate $\langle n \rangle$ can only reasonably be compared with N_{0A}, the number of pairs discovered by Arp and that comparison of N_0 with $\langle n \rangle$ is unfair, because N_0 includes QSOs found close to galaxies in other surveys and more than 200 tries are involved. If we take this more conservative point of view the result has

Table 1 All QSOs known to lie within 10 arc min of bright galaxies or their companions

QSO	Galaxy	Separation (arc s)	m_v (QSO)	z (QSO)	QSO	Galaxy	Separation (arc s)	m_v (QSO)	z (QSO)
UM228	NGC78B	516	17.0	0.102	UB1	NGC3384	265	19.4	1.111
UM245	NGC120	240	18.0	(1.46)	UB13	NGC3384	149	20.6	(0.497)
UM247	NGC132	300	17.0	2.35	4C61.20	NGC3407	173	16.3	0.422
BSO1	NGC157 (companion)	119	19.0	0.756	1107+036	Anon	20	18.9	0.96
UM268	NGC227	456	18.0	(1.66)	1108+289	NGC3561	66	19.0	2.192
PKS0038−019	NGC227 (companion)	67	18.0	1.690	BSO1	NGC3726 (companion)	100	18.4	1.13
PKS0038−020	NGC227 (companion)	~400	18.0	1.178	W1	NGC3992 (companion)	280	20.3	1.75
PKS0106+013	ZW0106.1+0123	192	18.39	2.107	1205−008	Anon	9.4	18.6	1.002
PKS0112−017	NGC448	600	18.0	1.365	3CR268.4	NGC4138	174	18.4	1.400
0121+108, MC2	Anon	40	18.0	0.510	MK205	NGC4319	42	14.5	0.070
UB1	NGC622	71	18.5	0.91	UB1	NGC4395 (companion)	145	18.7	1.265
BSO1	NGC622	73	20.2	1.46	B6	NGC4395 (companion)	329	18.4	1.038
PHL1226	IC1746	54	17.5	0.404	3CR270.1	NGC4395 (companion)	303	18.6	1.519
UB2	NGC772	352	19.4	2.61	UB1	NGC4395	370	19.2	0.77
0219+428	3C66B	390	15.2	(0.44)	Weedman 8	NGC4550	44	17.2	0.728
PKS0225−014	NGC936	600	18.0	2.037	3CR275.1	NGC4651	210	19.0	0.557
BSO1	NGC1073	104	19.8	1.945	1253+104 MC2	Anon	90	18.0	0.824
BSO2	NGC1073	117	18.8	0.599	UB1	NGC5055 (companion)	315	18.3	0.910
RSO	NGC1073	84	20.0	1.411	BSO1	NGC5296	55	19.3	0.963
UB1	NGC1087	170	19.1	2.147	UB2	NGC5660	483	17.3	0.205
4C−02.15	NGC1298	228	19.5	2.092	UB1	NGC5669 (companion)	70	17.7	0.766
0844+319	NGC2402	30	18.87	1.834	BSO1	NGC5682	95	19.2	1.94
0846+51	NGC2681 (companion)	12	19.5	1.860	1441+522	3C303	20	19.97	1.570
U2	NGC2859 (companion *A*)	66	(19.7)	2.25	3CR309.1	NGC5832	372	16.8	0.905
U3	NGC2859 (companion *B*)	73	(20.3)	1.46	BSO1	NGC5866	436	18.1	0.706
U1	NGC2859 (companion C)	60	(19.2)	0.23	UB1	NGC5981	107	19.0	2.132
0925+301	B20925+301	480	21.0	2.02	1548+114B	Anon	12	19.0	1.901
HOAG 1	M82	382	20.0	2.0492	W1	NGC6503	324	16.5	(0.76)
HOAG 2	M82	512	21.0	(2.1)	2020−370	Anon	21	17.5	1.048
HOAG 3	M82	585	21.0	2.0440	BSO1	IC1417	76	17.8	0.73
3C232	NGC3067	114	15.8	0.534	3CR455	NGC7413	24	19.7	0.543
UB2	NGC3338 (companion)	251	19.7	2.14	UB1	NGC7465	128	19.2	1.66
PKS1021−006	ZW1022.0−0036	126	18.22	2.547	UB1	NGC7592	415	18.7	1.410
					UB1	NGC7714−15	120	18.0	1.871
					UB2	NGC7714−15	480	19.0	2.193

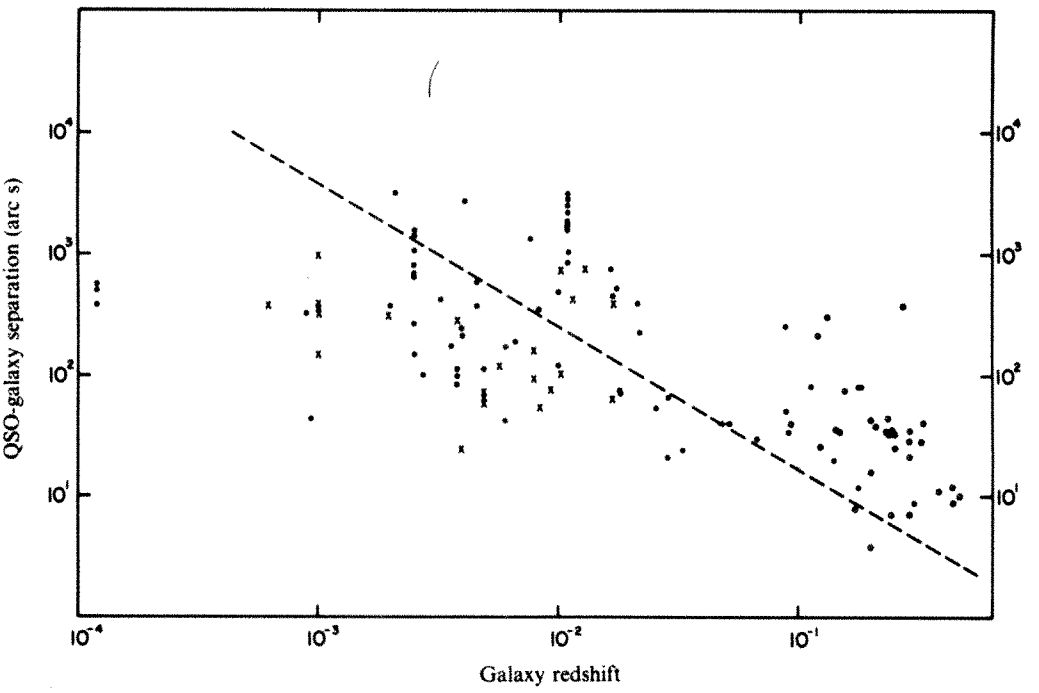


Fig. 1 A plot of log z_{galaxy} against log θ for all apparent QSO–galaxy associations listed by Hewitt and Burbidge[6]. The low redshift QSOs marked with a separate symbol are the QSOs close to galaxies investigated by Stockton[32]. The line is the least-squares solution which has the form log $\theta = -1.17$ log z_{galaxy} + constant. The range of slope at the 99% confidence level lies between −0.93 and −1.56. ●, Main galaxy; ×, redshift (main) versus θ (companion); ○, low redshift QSO.

obviously less statistical weight. Note that N_0 also includes the five 3CR QSOs found near to bright galaxies[5,8,15] which were shown to be highly significant[5] as a separate sample. These associations were found by studying galaxies in the vicinity of about 50 3CR QSOs. Thus only 50 tries were involved and our total of 200 tries assumed above can reasonably include that sample. Also included are the associations between Parkes QSOs and Zwicky catalogue galaxies[18]. Here about 90 QSOs were involved. The other sample which is included in N_0 are the cases recently found by Weedman[16] who has looked for associations between 259 QSOs found by objective prism techniques and 62 bright galaxies in the same area of the sky. He found that the apparent associations lay within one standard deviation of what one would expect by chance. The numbers that are seen as one goes to larger distances ($\sim > 5'$) are comparable with or even less than the numbers expected by chance. This may be because Arp has not searched as hard for QSOs further out. Alternatively it may be that the mean surface density of QSOs that we have used is too high. If the latter is correct, the result based on the number of close QSOs is enhanced.

What about NGC1073 where three QSOs are found to lie within 2′ of the centre of that galaxy. Let P_m be the probability that a single galaxy has m QSOs within θ arc by chance. Then

$$P_m(\mu) = \frac{\mu^m e^{-\mu}}{m!}, \qquad \mu = \frac{\langle n \rangle}{N}$$

From Table 2 we find that there are 34 galaxy–QSO pairs with separation $\leq 3'$. Because in the case of NGC1073 it was the first radio QSO which drew attention to the galaxy, we evaluate the probability that two QSOs are found within 3′ of a point which already has one QSO within 3′. Then $P = 0.0028$, and the probability that any one of the 34 galaxies with one QSO within 3′ will have two more there by accident is 0.095.

Alternatively we might ask what is the probability that any of the ~2,000 galaxies in the revised Shapely Ames catalogue has three QSOs lying within 3′ of its nucleus by chance. If $m = 3$, $P_m(\mu) = 7.22 \times 10^{-5}$. Thus the total probability is 0.144. This assumes that the vicinities of all of the bright galaxies have been searched for QSOs but only a very small number, perhaps 100, have been studied in this way.

Note also that the redshifts of the three QSOs associated with NGC1073 are 1.945, 0.599 and 1.411 (Table 1). This might be another accident, but it is striking that these values are exactly at the peaks of the redshift distribution discussed over many years (see ref. 17).

Many of the QSOs found near to bright galaxies by Arp and his colleagues are not close enough to be statistically significant if we estimate the probabilities in the normal way. But in many of these cases alignment of pairs of QSOs across the nucleus of a galaxy and the similarity of the redshifts of the QSOs makes physical association very likely. Alignments of this type include the QSO and two other compact radio sources associated with the radio galaxy B20925+30 (refs 4, 18), the QSOs around NGC3384 (ref. 19), QSOs around NGC2639 (ref. 20) and the pairs of aligned triple QSOs without a bright galaxy present found by Arp and Hazard[21]. All of the systems of this type have been listed elsewhere[6].

Given that there is some statistical evidence of association of galaxies and QSOs for close separations, and that alignments and similarity of redshifts of several pairs of QSOs around bright galaxies suggest physical association, we can look for further evidence by making a plot of the redshifts (proportional to distance) of the galaxies against angular separations of the QSOs from the galaxies. When this was originally done for the 3CR QSOs[8] it was found that the slope of the log z galaxy–log θ relationship was very close to −1, suggesting that the QSOs were at approximately a constant projected distance from the galaxies. Figure 1 shows a similar plot for all of the galaxy–QSO systems listed by Hewitt and Burbidge. Included are 94 QSOs associated with 65 galaxies. Obviously some of these may be accidental and because in many cases several QSOs at different distances are associated with the same galaxy, we expect considerable scatter in the relationship. However, a least squares solution gives a best fit line for log z given log θ of the form

$$\log \theta = -1.17 \log z + \text{constant}$$

The range of slope for this line at the 99% confidence level is between −0.93 and −1.56. The correlation coefficient associated with the (log θ, log z) pairs is $r = 0.68$. This correlation is significant.

Pairs of QSOs

Stockton[22] found the first close pair of QSOs separated by 35″, with very different redshifts. He calculated *a posteriori* that the probability that this was due to chance was 6×10^{-3}. The next pair that was found was 1548+114a and b, with a separation of 5″ (ref. 23), *a posteriori* probability calculation gave the probability of a chance superposition $\sim10^{-2}$. Bolton *et al.*[24] carried out a systematic search of 100 fields centred on flat spectrum radio-emitting QSOs from the Parkes 2,700 MHz survey. They found eight candidate QSOs within 1.7′ of the radio-emitting QSOs, and have confirmed that there are at least four and probably five pairs of which at least four and probably five pairs have different redshifts. As the plate limit in these studies was $B=19$–19.5 mag, the number of QSOs per square degree is about three, and thus the number of pairs expected by chance is 0.76. Since four or five pairs are found, Bolton *et al.* consider that the probability that this is due to chance lies in the range of 6×10^{-3}–10^{-4}. Wills and Wills[12] then did a similar survey in the north, searching within 2′ of 100 QSOs identified in the NRAO 5,000 MHz surveys. They found two pairs and one is expected by chance. Thus from the two fields together, seven pairs have been found against the 1.76 expected by chance.

Several new close pairs or triplets have recently been found. They include a pair with separation 5.7″ with precisely the same redshift[25] which must be a physical pair, or a single object which appears double due to an intervening massive object[25], and the triple system of QSOs near M82[26] with separations of 75″ and 125″. Each QSO has a redshift close to 2.04.

Perhaps we are seeing several phenomena in the close groups, including chance effects, physical pairs with the same redshift, physical pairs with different redshifts, and physical pairs or triplets with similar large redshifts ejected from nearby bright galaxies. Only further observations will clarify this situation.

Associations between QSOs and galaxies at the same redshift

The first successful attempt to find a galaxy at the same redshift as a QSO was made by Gunn[27], and Robinson and Wampler[28] who found one or two normal galaxies lying near the QSO PKS2251+11. One at least has a redshift very close to 0.323 which is the redshift of PKS2251+11. Gunn made statistical arguments *a posteriori*, concluding that there was a very low probability that this association was accidental and this result was heralded as evidence that the redshifts of the QSOs are of cosmological origin. Several other similar cases were found[29–32]. However, there was no reliable way of estimating the statistical significance of these associations[33].

Recently Stockton[7] has carried out a much more significant study. He chose all of the known QSOs (27) with redshifts ≤0.45, $m_v<19.2+5\log z$ and at declinations $-15<\delta+55$ and attempted to obtain spectra of all galaxies visible on the red Palomar Sky Survey plates lying within 45″ of the QSOs. Of the 29 galaxies he identified, he obtained spectra of 25 and has reported that 13 galaxies in eight fields (out of 27) have redshifts, cz, within 1,000 km s^{-1} of the redshift reported for the QSO. He has estimated that the probability that this could occur by chance is less than 1.5×10^{-6}. Stockton[30] also has found a galaxy at the same redshift as 3C273, although it lies further away than 45″.

These results provide strong evidence that some QSOs at least lie at cosmological distances.

General studies of correlations between faint galaxies samples and QSOs

Several attempts have been made to look for correlations between the positions of QSOs and faint galaxies contained in the Abell catalogue and the Lick catalogues. The maximum redshift for any of these galaxies is $z\sim0.2$.

Bogart and Wagoner[34] found marginal but not convincing evidence for an apparent correlation between the positions of QSOs with large redshifts ($z\geq0.3$) and Abell clusters ($z\leq0.2$). Recently Roberts *et al.*[35] investigated the positions of all QSOs with measured redshifts (498 objects) in the area of the sky containing the Abell catalogue of rich clusters. It was found that there is no statistically significant evidence that either low- or high-redshift QSOs lie preferentially close to Abell clusters. This is in contrast to the highly significant evidence that 3CR radio galaxies lie preferentially close to Abell clusters.

This result probably supersedes the early result of Bogart and Wagoner because much larger samples are involved. It does not contradict the work of Stockton as the galaxies he found close to his sample of low redshift QSOs are rarely, if ever, in rich clusters.

Seldner and Peebles[36] have found statistically significant evidence of a correlation between the position of 382 QSOs at $|b|\geq40°$ $\delta>-23°$ and the Lick counts of galaxies, $m\leq19$. They find on the average 1.45 ± 0.39 more galaxies within 15′ of a QSO than would be expected if the QSOs were placed at random. There seems to be no trend with QSO redshift or apparent magnitude and the effect is not apparently due to a few QSOs at the same redshifts or the galaxies of the type found by Stockton. Again this result does not conflict with the absence of correlations with Abell clusters as the majority of the galaxies in the Lick counts lie in small groups and more extended distributions.

From these results and those of Stockton we conclude: first, that QSOs are not present in rich clusters; second that QSOs are sometimes physically associated with small groups of galaxies. In some cases they may have the same redshifts, with no significant non-cosmological component, while in others they may be a large non-cosmological component.

Conclusion

Based on the evidence discussed here some QSOs apparently have redshifts which are completely cosmological in origin, but others have redshifts of largely non-cosmological origin. It is hard to deny either the evidence of Stockton, or the evidence of QSOs found close to bright galaxies or their companions.

The results therefore imply either that there are two distinct classes of QSOs with very different physical redshift mechanisms operating, or that the Doppler effect is still always responsible. In this latter situation both cosmological Doppler shifts and those due to high speed ejection of QSOs from galaxies must be involved.

Table 2 Comparison between the numbers of QSOs found near to bright galaxies (N_O or N_{OA} and the number expected by chance ($\langle n\rangle$))

Apparent magnitude	N_O	$\theta\leq60''$ $\langle n\rangle$	N_{OA}	N_O	$61''\leq\theta\leq120''$ $\langle n\rangle$	N_{OA}	N_O	$121''\leq\theta\leq180''$ $\langle n\rangle$	N_{OA}	$N_O\langle n\rangle$	$181''\leq\theta\leq300''$ N_{OA}	
≤17	1	0.05	0	1	0.16	0	1	0.24	1	1	0.82	0
≤18	5	0.17	0	6	0.52	4	1	0.86	1	2	2.8	0
≤19	9	0.52	1	12	1.55	9	4	2.4	2	4	8.2	0
≤20	12	1.73	3	16	5.2	12	6	8.6	4	7	27.6	2

Apparent magnitude	N_O	$301''\leq\theta\leq600''$ $\langle n\rangle$	N_{OA}	N_O	$\theta\leq180''$ $\langle n\rangle$	N_{OA}	N_O	$\theta\leq600''$ $\langle n\rangle$	N_{OA}
≤17	4	3.9	1	3	0.46	1	8	5.2	2
≤18	9	13.0	2	12	1.56	5	23	17.3	7
≤19	15	39.1	8	25	4.62	12	42	51.9	20
≤20	18	129.6	10	34	15.5	19	59	173	31

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articles

A theory of terrestrial catastrophism

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The time sequence of terrestrial catastrophes and other Solar System phenomena is apparently stochastic but with an underlying galactic modulation. A mechanism is proposed involving the capture of planetesimals as the Sun passes through spiral arms.

THE radial separation of galactic spiral arms and the epicyclic frequency of the solar orbit imply quasiperiodic intervals between crossings of a few 10^7 yr. Should galactic evolution involve the formation of successive generations of spiral arms (see ref. 1), the intervals will be modulated by an additional quasiperiodicity of a few 10^8 yr. If spiral arms were to contain planetesimals, each Solar System passage would lead to the capture and formation of a temporary 'Oort cloud' which would be rapidly dissipated by planetary encounters and hyperbolic ejection. We shall argue that the cratering flux record, the current distribution of comets and the existence of many dynamically short-lived bodies in the Solar System, are each best explained as consequences of this single mechanism. With this viewpoint, comets and most asteroids and satellites are temporary survivors of one or more capture episodes. The impacting planetesimals can be shown also to cause, *inter alia*, water erosion on Mars, intermittent biostratigraphically recognisable events in Earth history (including mass extinctions) and ice ages. The latest rapidly declining series of events follows our passage through the Orion spiral arm. As the Gould Belt complex, at a mean distance of ~250 pc, is the nearest appendage of the Orion arm to the Sun (see ref. 2), and has a relative velocity of ~25 km s^{-1}, this capture episode ended ≤10 Myr ago. The most recent spell of ice ages, within the past ~20 Myr was preceded by a more temperate period[3] lasting ~40 Myr. Furthermore, assuming the Orion arm has a typical mass content (~10^6–10^7 $M_\odot$ kpc^{-1}), the calculated remaining flux of low mass planetesimals agrees with the observed 'short period' (Apollo) asteroids and an impact rate inferred from very recent terrestrial events.

Cratering flux history

The record of impact cratering on the Moon and inner planets[4] shows the planetesimal flux to have declined steeply for the first 2,000 Myr of the Solar System's existence, and then to have attained a constant value over the past 2,500–3,500 Myr. Superimposed on this trend are quasiperiodic bombardments, of uncertain magnitude and duration, separated by time intervals characteristically a few 10^8 yr. A similar periodicity is evident in the deposition of micrometeorites on lunar soil[5].

Dynamical studies[6] indicate that the steeply declining phase of the cratering history can be understood in terms of a sweeping-up, or hyperbolic ejection, of primordial debris by the planets. Rapid depletion of the inner Solar System debris occurs so that after ~10^8 yr, bodies entering the inner Solar System have semi-major axes primarily ~10^5 AU. The existence of these high initial flux rates is clear evidence that the Solar System has been immersed in a cloud of planetesimals in the past.

Two important features of the cratering history remain to be explained: the brief periods of enhanced bombardment; and the remarkable constancy, in the mean, of the planetesimal flux taken over the past ~3,000 Myr.

There are numerous perturbing sources in the interstellar medium. Thus the Solar System has passed through ~15 nebulae of radii a few pc and $n_H > 10^3$ in the past 4,500 Myr, and experienced ~60 close encounters (say with impact parameter $p < 8$ pc) over the same period[7]. Such nebulae have masses $M \sim 10^4 M_\odot$, and encounters with them would result in drastic perturbations of the planetesimal cloud and consequent flooding of the inner Solar System. This could account for the bombardment episodes. However, the very efficacy of the process raises

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Table 1 Percentage of outer Oort cloud planetesimals ejected through close passage of a massive nebula*

M (10^4 $M_\odot$)	p (pc)	V (km s^{-1})	%
1	5	30	36
2	5	30	50
1	2	10	90

* Based on three-body numerical integrations (program by M. Staniucha).

difficulties for the survival of a cloud virtually intact for half the lifetime of the Solar System, as is implied by the constant flux.

Numerical trials have been run in each of which 2,500 massless bodies bound to the Sun were perturbed by a passing nebula idealised as a point mass of prescribed (M, p, V), where V represents the flyby velocity. The orbits were randomly orientated and the semi-major axes a lay in the range $10^4 \leq a \leq 2\times10^5$ AU, with eccentricities e in the range $0 \leq e < 1$: the test particles were therefore on the outer fringes of the hypothetical Oort cloud. The results of the three-body integrations are shown in Table 1. Clearly the outer part of the Oort cloud, from which long-period comets are supposed to derive, would be very efficiently cleared by passing dense nebulae, and it does not seem likely that the comet-producing regions of the cloud could survive ~30 such encounters, let alone yield a constant cratering flux for the past ~3,000 Myr.

Even if we disregard these difficulties, the hypothetical Oort cloud as currently envisaged is unlikely to be the source of impacting planetesimals. Thus, its mass is supposed to be a few $m_\oplus$ based on a comet mass of $\sim10^{17}$ g and the likely number of comets[8,9]. The crater size distribution[10] reveals a mass function $\propto m^{-1.6}$ with a typical upper limit of $m > 10^{20}$ g (for example the Tycho crater was formed ~400 Myr ago[11] by a mass of $\sim10^{19}$ g). The bulk of the mass of the system must therefore be concentrated in the few largest bodies whereas the 'characteristic mass' of a comet is inferred from the more common bodies at the low-mass end of the spectrum. Note that some observers[12] give independent reasons for supposing a maximum cometary mass $\sim10^{20}$ g. It follows that the mean mass of a comet should be increased by a factor ~40 giving 80 $m_\oplus$, say, for the present Oort cloud. The process which fed comets into the Oort cloud would have thrown the bulk of the material into hyperbolic orbits[13]. Thus 80 $m_\oplus$ represents a total ejection of >1,300 $m_\oplus$ and probably >2,700 $m_\oplus$. These amounts are much too large to be dynamically possible for the current Solar System configuration.

These results indicate that the cratering flux must comprise two components: one a decaying part due to the primordial swarm of bodies bound to the Sun, the other a constant part which has become dominant over the past ~3,000 Myr as the first decayed. The main belt asteroids fail by a factor of ~10 to supply the impacting planetesimals[14] and would in any case be a decaying source. The only other possible location for the constant flux source is interstellar space. In this view, the long period comets have to be identified as the most easily visible component of the captured planetesimals. (The idea that comets are interstellar in origin was first suggested by Laplace[15].) The fact that there are few certain hyperbolic orbits amongst the long period comets indicates the Sun is not currently immersed in a significant interstellar source[16]. The observed flux is thus the residue of captures effected in the recent past.

The hypothetical Oort cloud should be thermalised by weak stellar encounters[17] on a time scale ~1 Myr. The perihelia q should thus be uniformly distributed both over the sky and in distance from the Sun. The perihelion distribution of long period comets in fact increases as $q^{1/2}$ in the inner Solar System[18], lies in a preferred plane ~20° from the Milky Way, and concentrates ~7° from the solar apex[19]. There is thus good evidence amongst the comets favouring a capture mechanism related to the Sun's motion through the local galactic environment.

Probably the dominant capture mechanism is through entry of planetesimals into Jupiter's sphere of influence: thus Radzievskii and Tomanov[20] have shown that Jupiter can capture $\sim10^5$ planetesimals of mean mass 10^{15} g during the passage, lasting 0.1 Myr, through a cloud whose planetesimal component has a smoothed-out mean density 10^{-20}–10^{-22} g cm^{-3}. For mean mass 4×10^{17} g the required densities are 4×10^{-18}–4×10^{-20} g cm^{-3}. These densities are high but not unreasonable; and if planetesimals are widely distributed through spiral arms rather than concentrated in dense clumps, the requisite densities are correspondingly relaxed.

Yabushita[21] has shown that the energy distribution of comets in the Solar System evolves to a form which, after 3–6 Myr, is almost independent of the initial distribution and is concentrated in the range $2\times10^4 < a < \infty$ AU. To be consistent with observation, capture must have taken place between 3.6 and ~9 Myr ago. Within the errors, this agrees satisfactorily with the emergence ≤10 Myr ago of the Sun from Gould's Belt.

We conclude that the constant average flux of planetesimals during the past ~3,000 Myr is ultimately of interstellar origin, and that the fluctuations in the bombardment rate reflect passage through successive spiral arms, the most recent of which is responsible for the currently observed declining flux of comets.

Some implications of interstellar planetesimals

If our hypothesis is correct, then planetesimals, possibly up to at least lunar size, must occur in abundance in spiral arms. Note that a depletion of C, N and O observed in the interstellar medium has been ascribed to the existence of interstellar comets[22] and has certain implications.

First theories of the origin of the Solar System involving accumulation of blobs rather than, say, condensation of a solar nebula, become more plausible. Star formation as a process of aggregating small masses rather than fragmenting large ones is then probable. Although such ideas[23,24] have never found favour, the alternative of Jeans collapse is proving unsatisfactory[25]. The hypothesis is *a priori* consistent with star formation occurring predominantly in spiral arms. The planetesimal mass function indicated by the crater size distribution is developed in a fragmenting–accreting regime[26] and must be consistent with the presence of dust in spiral arms. Cold 'globules' have been observed[27], and these provide evidence that dusty protostellar objects of the kind implicit in this scheme of star formation do actually exist. These objects are also notable sources of CO emission[28], and not only is CO emission generally very concentrated in the galactic plane where spiral arms lie[29], but it is commonly detected in regions of intense star formation[30]. We emphasise therefore that although the existence of planetisimals is not generally considered to be an essential requirement for any theory of how stars are formed, it is, nevertheless, consistent with observation.

A deeper problem arises when we consider the origin of the planetesimal material. The interstellar accretion processes stu-

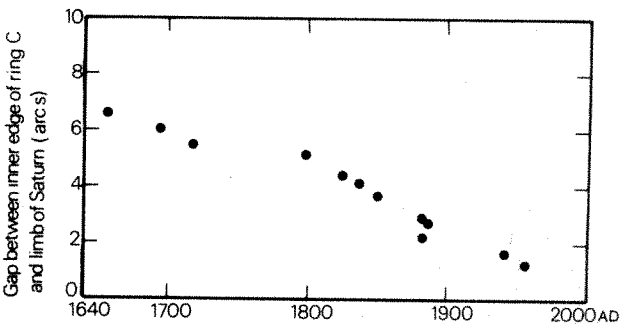


Fig 1 Gap between inner edge of ring C and limb of Saturn, measured in arc s and normalised to a mean opposition. Secular evolution of the whole ring system on a time scale a few 10^4 yr seems probable.

Table 2 Short-lived or recent Solar System phenomena

Object	Phenomenon	Time scale (yr)	Probability*	Comments	Refs
Chiron	Hyperbolic ejection from Solar System	$\sim 5\times10^{4}$	$\sim10^{-5}$		53, 54
Hidalgo	Hyperbolic ejection from Solar System	$\sim10^{6}$	$\sim2\times10^{-4}$		55
Triton	Spiralling in towards planet	10^{7}–10^{8}	10^{-3}–10^{-2}	Largest satellite (~3,700 km diameter); retrograde orbit, indicative of capture	36, 37
Phobos	Spiralling in towards planet	$\sim6\times10^{7}$	$\sim10^{-2}$		38
Rings of Saturn	Secular evolution	$\sim10^{2}$–10^{4}	$\sim10^{-6}$	See Fig. 1. Ring C is spreading inwards at ~1.6 arc s per yr	39–41
Mimas/Tethys resonance	Evolving	$\sim2\times10^{8}$	$\sim4\times10^{-2}$	2:3 resonance in period, disturbed or formed a few 10^{8} yr BP	42
Enceladus/Dione resonance	Evolving	$\sim4.4\times10^{8}$	$\sim10^{-1}$		43
Outer retrograde jovian satellites	Temporary capture	?	?	Orbits are retrograde, indicative of capture, and are unstable in Hill's sense; asteroids are an inadequate replenishing source	44–48†

* Probability is time scale divided by 4.5×10^{9} yr.
† A. E. Roy, personal communication.

died so far[31] give growth times of ~100 Myr to attain particle diameters $\sim10^{-5}$ cm. Some forced diffusion mechanism (see refs 32, 33) of remarkable efficacy is required if, say, density waves were the prime agency in forming spiral arms. But if spiral arms were the result of some exotic process, say involving recurrent (galactic) nuclear explosions, then one might look for the origin of planetesimals in that same exotic process. Seyfert events may be the result of such explosions at 10^{8} yr intervals[34], and these can be understood in terms of very massive stars ($\geq10^{8}\,M_{\odot}$) evolving through and beyond a nuclear burning phase[35]. On this hypothesis therefore, planetesimals are suddenly cooled fragments of central highly evolved, very massive stars[1].

Recent events and anomalous phenomena in the Solar System

One might expect to find manifestations of interstellar capture events among the small body population of the Solar System. Indeed we propose that in addition to the comets, many asteroids and satellites form a temporary population in a state of relatively rapid (10–100 Myr) dynamic evolution, being the survivors of one or more capture episodes.

Table 2 lists some short-lived Solar System phenomena. Qualitatively, these find a unified explanation in terms of a temporary capture scenario; taken cumulatively, they seem otherwise inexplicable. For example the probability, on a conservative view, that we happen to simultaneously observe the Triton, Phobos and Chiron events is $\sim2\times10^{-10}$. Singer[49] has argued that all the regular satellites of the major planets were captured after the formation of the planets themselves. Currently, direct orbits predominate because retrograde orbits decay more rapidly. The slope of the satellite mass function is similar to that of asteroids, in agreement with a common origin hypothesis. The largest satellite masses are also close to that which comes within the capture radius of Jupiter during spiral arm passages.

A fundamental problem of the asteroid system is that it has much more internal kinetic energy than can be fed into it by jovian perturbations. Jupiter can induce eccentricities of at most a few hundredths in the asteroid system, substantially less than those observed (which are up to ~0.40)[50]. Another manifestation of this problem[51] results from application of Tisserand's criterion to Pallas: it has been shown that this large asteroid (and others) cannot have been perturbed by Jupiter from the invariable plane into its high-inclination orbit—yet conventionally, accretion could have occurred only in the quiescent conditions supposed to pertain in the invariable plane[52].

These problems do not arise on the capture hypothesis, according to which the asteroid zone represents a relatively stable region of phase space, and the asteroids are, in part or whole, interstellar planetesimals which have accumulated during successive bombardment episodes. One therefore expects the asteroids to occupy every available stable niche of phase space, as is observed. The observed chemical differentiation is then a matter for conjecture: the outer, carbonaceous asteroids may simply not yet have lost their surface coating; or we may be observing the products of successive capture events from interstellar regions with locally different properties.

Short-period comets are regarded as deriving from intermediate and long-period comets through jovian perturbations[56]. As they have lifetimes of only a few centuries then one expects dynamic equilibrium to be established in time scales of that order. A recent study[18] of the number density of long-period comets in the Solar System indicates that this is constant throughout the inner regions, and is ~0.09 AU^{-3}. With this figure only 1 comet per 11 yr is expected to enter the 'capture region' around Jupiter. The probability that a comet, entering the capture region, will actually be captured[57], is $\sim7\times10^{-3}$. One therefore expects an interval ~1,600 yr between captures of long-period comets into short-period orbits. Since the lifetime[58] before disintegration of a short-period comet is ~500 yr, then there is an expectation on the 'steady state' model of observing ~0.3 short-period comets. The number actually observed ~80.

The explanation may be that a moderately large comet (say diameter 20 km) disintegrated due to tidal forces while in the process of being captured into a short-period orbit. The maximum fragment size would be determined by the tensile strength of ice and the closeness of passage to Jupiter: fragmentation into say $\sim10^{3}$ pieces of diameters ≤2 km is feasible.

This discussion implies that the intermittent capture hypothesis provides a single, simple explanation of diverse phenomena which otherwise require a variety of *ad hoc* theories.

Planetary impacts

The terrestrial cratering history of the Phanerozoic[10] indicates that the smoothed interval between formation of craters of diameter $D\geq20$ km, is $\delta t\simeq5.7\times10^{5}\ (D/20)^{2}$ yr, with D

measured in kilometres. The kinetic energy E of impact is related to D by $E = 2.4\,D^{3.4}$, valid for $D \geq 14$ km. (It is convenient here to measure energy in megatons equivalent of TNT: one megaton $\equiv 1$ MT $= 4.2 \times 10^{22}$ erg.) Given E, a density of 3.3 g cm^{-2} and a r.m.s. impact speed of 24.6 km s^{-1}, the projectile diameter d follows. Hence (δt, d, E) can be calculated in terms of D and are listed in Table 3.

The actual frequency of impact is bunched into episodes of spiral arm immersion; hence from Table 3, the Earth is expected to suffer one impact of energy at least 1.6×10^8 MT during each passage of the Sun through a spiral arm; an impact with $E > 5 \times 10^8$ MT during a passage is expected with probability ~0.5; and an impact of energy $\geq 10^9$ MT is expected sometime during the Phanerozoic. These intervals should be compared, for example, with the mean duration of a biostratigraphic period (~50 Myr). These large impacts must inevitably have global consequences, directly through the effects of blast (see ref. 59), and indirectly through the injection of dust[60] and NO into the stratosphere.

For land impacts, the smallest overpressure likely to be experienced anywhere on Earth is ~0.25 kg wt cm^{-2} when, for example, $E \sim 5 \times 10^8$ MT. Peak overpressures of this magnitude completely destroy forests[61]. As large land vertebrates of small strength/weight ratio would be preferentially killed[62], such a blast wave could have been the prime cause of the Cretaceous–Tertiary extinction[63,80]. The survival of animal life associated with underground habitats and the rapid regeneration of vegetation from roots and seeds would be consistent with this hypothesis. An impact of this energy would also excavate $\sim 10^{18}$ g of material much of which would reach the stratosphere as a fine dust and block out sunlight, with catastrophic effects on food chains[63]. If the settling time were ≥ 10 yr, the build up of ice could be ~0.3 km in depth and continental glaciation might be initiated[64]. Analogy with nuclear explosions suggests that $\geq 10^{15}$ g of NO would be injected into the stratosphere[66], thereby destroying the ozone layer. The biological effects of the UV deluge to be expected as the sky clears are likely to be drastic[67]. Ocean impacts, causing waves up to 8 km amplitude, will generally be more common than land impacts, and through simultaneous flooding of the land and obscuration of the upper atmosphere are likely to be an extremely efficient cause of sudden ice ages. An impact in the Palaeozoic Pacific at the close of the Frasnian explains[68] all the major aspects of an event which included the sudden extinction of shallow sea life.

Impacts of the sort expected at ~50 Myr intervals therefore produce an abundance of deleterious effects, the consequences of which are catastrophic extinctions. The situation is likely to be more complex for lesser impacts but the gradual extinction of taxa might be initiated indirectly: thus the widespread brachiopod extinctions near the Ordovician–Silurian boundary are correlated with general glaciation and withdrawal of the sea from continental platforms[69]. A quasi-periodicity in the numbers of taxa is clearly evident[70] and is ~50 Myr. Geophysical consequences are more conjectural, but the correlation between extinction events, field reversals and rotation period[71] may have impacts as a common cause. It is possible that frequent ice ages so alter the mass distribution on plates as to raise or lower land levels[72], precipitate orogenic activity at their perimeters, and even provide a driving force for tectonics. Urey[73] suggested that impacting comets might induce the termination of geological periods and pointed out a correlation with tektite ages. Table 4 lists the ages of the known tektites, and all known craters of diameter >50 km, against the ages of the geological periods. As the record must be grossly incomplete, it is surprising that, within the uncertainties, tentative correlations can be made, agreement becoming poorer the more remote the epoch.

Table 3 Interval δt between impacts with planetesimals of diameter $\geq d$, with impact energies $\geq E$ and producing craters of diameter $\geq D$

D (km)	δt (yr)	E (MT)	d (km)
80	9.2×10^6	7.1×10^6	3.9
100	1.4×10^7	1.5×10^7	5.0
200	5.8×10^7	1.6×10^8	10.9
500	3.6×10^8	3.6×10^9	30.8

Table 4 Ages of geological periods compared with tektite ages and ages of all known Phanerozoic craters > 50 km diameter

Biostratigraphic interval	Age at beginning (Myr)	Tektite/ crater age (Myr)	Tektite/ crater
Pleistocene	1	0.77 ± 0.10	Australites
		0.88 ± 0.93	Ivory Coast
Pliocene	13	14.7 ± 0.7	Moldavites
Miocene	25	28.6 ± 2	Libyan desert glass
Oligocene	36	34.7 ± 2	Bediasites
		38 ± 9	Popigai, 100 km, USSR
Eocene	58	57	Kara, 50 km USSR
Palaeocene	63	?	?
Cretaceous	135	?	?
Jurassic	181	183 ± 3	Puchezh-Katunki, 80 km, USSR
Triassic	230	210 ± 4	Manicougan, 70 km, Canada
Permian	280	?	?
Carboniferous	345	365 ± 7	Siljan, 52 km, Sweden
Devonian	405	?	?

Comets may comprise ~40% water ice by mass[74], and this may hold for a proportion of the larger planetesimals: the low densities of the saturnian satellites, for example, may indicate an icy constitution. The impact hypothesis therefore offers a natural explanation for the extensive water erosion on Mars, which is known to have arisen from one or more catastrophic flooding events[75].

A prediction of the hypothesis is that anomalous isotopic and abundance anomalies should occur, because of global dust settling, at biostratigraphic boundaries. Thus Pt, Ir and Os are typically 10^3 times more abundant in extra-terrestrial than crustal material. W. Alvarez *et al.* (personal communication) have found that, in a 1-cm clay layer marking the Cretaceous–Tertiary boundary at Gubbio, Italy, the Ir abundance jumps by a factor ~20, decaying upwards with a half height of 8 cm. The absence of ^{244}Pu, at ~0.1 of the level predicted by nucleosynthetic theories, seems to preclude a supernova source.

Discussion

Clearly the picture presented here needs clarifying. In particular the implications of large amounts of planetesimal material in spiral arms, and the terrestrial consequences of large impacts, need to be explored further. Nevertheless, starting from (and justifying) the interstellar capture hypothesis, we have been able to unify a wide range of galactic, Solar System and terrestrial phenomena; and we have identified the most recent capture event as having ended ≤ 10 Myr ago with the emergence of the Solar System from Gould's Belt.

Our theory enables the flux of planetesimals on the Earth at present, corresponding essentially to the short period core of the Apollo asteroids (ref. 14) to be calculated. This flux results in one $10^{3\pm1}$ MT event every ~10^4 yr and one of these may well have been responsible for initiating the short ice age from which Earth emerged some 10^4 yr ago. (There is a 20% probability that the event lies in the upper half of the stated range.) For comparison, the Krakatoa volcano[76] had an energy of 50 MT and the Tunguska meteorite[77] ~10 MT: these caused ~10% and ~1% drops in the solar constant respectively over ~1 yr. A stochastic distribution of similar ice ages over the preceding ~10^7 yr is implied, although with additional modulation by the Milankovitch process (see ref. 78). With the usual planetesimal mass function, the flux also corresponds to one $10^{2\pm1}$ MT event per ~1,000 yr, each with possibly detectable consequences.

Thus, we might expect these to produce a temporary hemispheric climatic cooling and, following calculations by Brown and Hughes[79], a correlated enhancement of ^{14}C. Discrepancies in ^{14}C chronology during the past several thousand years are known to occur and may coincide with 'mini' ice ages[65]: a common cause for such effects seems probable. Planetesimal impacts have not been previously suspected in this context, and yet their impact frequency is apparently such that historical records are a potential source of data for testing our hypothesis.

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Cooperative effects in simulated water

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Computer simulation calculations on liquid water, aqueous solutions and biological interfaces have been made almost universally assuming pair-additive interactions. Experimental and theoretical evidence implies this is a serious oversimplification. The development and testing of a different approach is reviewed in which many-body forces are specifically taken into account through molecular polarisation. Solution and interfacial properties of aqueous systems are particularly sensitive to such forces.

DURING the past decade there have been significant advances in our understanding of the molecular structure and dynamics of the liquid state. This has been due largely to the development of computer simulation techniques such as Monte Carlo and molecular dynamics methods[1–3] by which we avoid the problems remaining in analytical theories of simple liquids[4].

The success of these methods applied to the simple liquids of the theoretician (such as liquid argon) has encouraged applications to more complex liquids. In particular, the universal relevance of water in chemistry and biology, coupled with the fascination of its 'abnormal properties', have made aqueous systems a popular target for simulation methods[5–9]. But if results are to have more than a hypothetical relevance to the real liquid, the potential we use must model adequately the behaviour of real water molecules. We discuss some of the difficulties facing realistic simulations of 'water-like water' which are unavoidable if solution effects and the operation of biological systems are to be understood properly. We pay special attention to the nature of water–water hydrogen bonding and argue that the long suspected cooperative nature of this interaction[10] must be taken into account.

Pair additivity

A variety of potentials has been used to model the water–water hydrogen bonding interaction, such as the four-point-charge ST2 (ref. 11) potential which was derived from an earlier effective pair potential (BNS)[12], which was itself fitted to the measured second virial coefficient of steam. Other potentials in use include central-force models (CF) such as that of Lemberg and Stillinger[9] which treats the hydrogens and oxygens as separate charged units, and one developed by Clementi by

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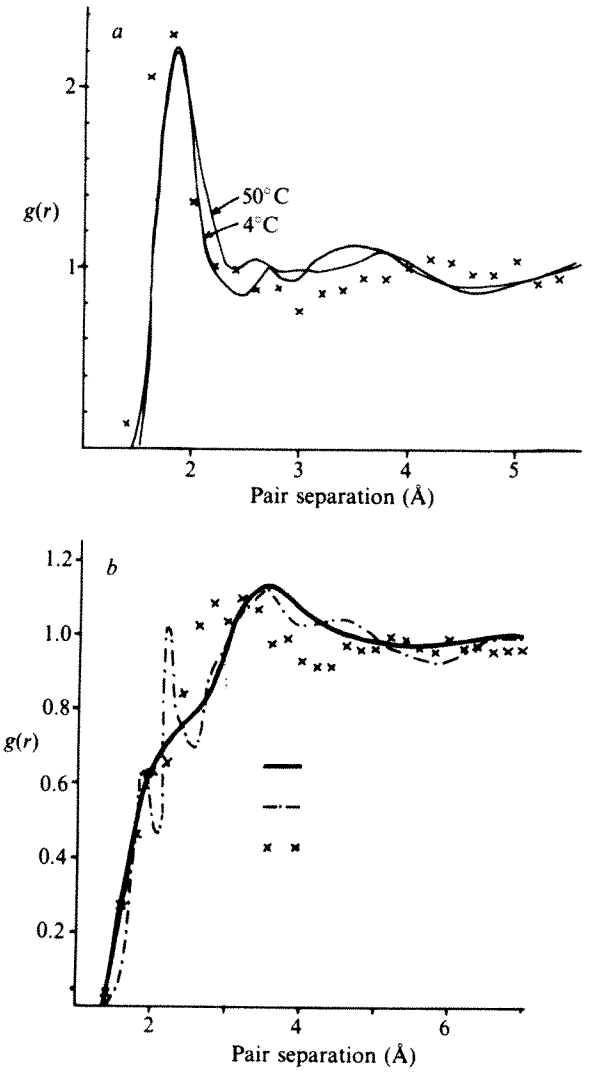


Fig. 1 *a*, Pair distribution functions, $g(r)$, obtained from X-ray scattering data[46] on water at 4 °C and 50 °C (——) and from simulated PE-water (×). The starting configurations were generated from an assembly of 216 molecules (previously equilibrated under the ST2 potential) with 40,000 Monte Carlo steps using a cubic box length of 18.62 Å, normal periodic boundaries and a cut off of 7–7.5 Å. *b*, A comparison of the neutron pair distribution function of D_2O at 11 °C (——) with simulation predictions using the central-force (CF) model of Stillinger and Rahman[51] (- · - ·) and the polarisable electropole (PE) model (×). The PE model reproduces the low-r shoulder and is notably free of the strong peaking incorrectly predicted by the central force model (which indicates the over-tetrahedrality of the latter potential).

fitting an analytical form to quantum mechanical calculations on the water dimer[7].

All these potentials are pair-additive: the energy of a given assembly is expressed as a sum of interaction energies between all pairs of molecules in the assembly. In other words, the interaction between an isolated pair of molecules is assumed to be unaffected by the intrusion of a third molecule. This is a universal assumption when dealing with simple liquids, where it is an excellent approximation. For water it has been argued that non-pair-additive effects, although accepted to be greater than in simple liquids, can be either neglected or somehow averaged[12].

However, it has been stressed[13] that none of these pair potentials can account for both gas and condensed phase properties of water, and recent neutron diffraction data[14,15] on D_2O demonstrates that current four-point charge and central-force models predict an overstructured liquid. Moreover, the known failure of these simple models to account for dielectric properties is a serious drawback to understanding solvent effects in real aqueous chemical or biological systems. The following observations underline the inadequacy of pair-additive potentials: (1) Isolated water molecules are poor hydrogen donors and acceptors; in the gas phase, water is less acidic than toluene, less basic than formic acid[16]. This strongly implies that its celebrated acid/base properties must relate to its bulk phase, rather than to the isolated molecule. (2) Quantum mechanical calculations on, for example, water trimers and tetramers, suggest that hydrogen bonding in these clusters is stronger than in the dimer by 20–30%[17,18]. (3) The dipole moment of the isolated water molecule is 1.85 D (ref. 19), yet the average value for hexagonal ice I is between 2.6 and 3.0 D (ref. 20). Such a large dipole moment enhancement is highly relevant to dielectric and solution properties.

One might argue that an effective pair potential might be constructed to reproduce such condensed phase effects; in view of the known extent of the non-pair additivity, such a procedure is not only artificial but is incapable of reconciling gas and liquid phase properties and therefore most unlikely to be able to predict localised effects such as at interfaces. This evidence forces us to take the cooperative effects into account specifically, and to examine their consequences.

The polarisable electropole water model

The electronic charge distribution of an isolated water molecule is represented by a multipole expansion as described by Buckingham[23] using the known dipole and quadrupole moments plus a spherically symmetric Lennard–Jones function. In developing a polarisable electropole (PE) water model[21,22], an electropole formalism (see Table 1 legend) was chosen in preference to a four-point charge model, (1) to avoid the need for artificial switching devices[11] necessary to prevent charge overlaps, (2) to ensure the correct sign and magnitude of the quadrupole moments (ST2, for example, yields a quadrupole moment of the wrong sign), and (3) to minimise the need to fit adjustable parameters. The non-pair additivity is handled using a classical polarisability tensor.

The use of this classical device requires explanation, considering that any significant covalency would result in appreciable charge transfer between two hydrogen-bonded molecules. However, as the size of the basis set used in *ab initio* quantum mechanical calculations on the water dimer is increased, the amount of charge transfer predicted falls to a degree where electronic cloud distortion into the hydrogen bond region is high but actual intermolecular electron transfer is small[28]. A polarisation algorithm can reproduce the major effects of this charge distortion.

In performing polarisation calculations, the resultant electric (dipole + quadrupole) field acting on a given molecule will induce an electron cloud distortion quantified by the product of the field and the polarisability. This polarisation gives rise to corrections to the previously calculated fields and hence to a further change in the calculated polarisation, and so on. The total polarisation is thus determined in an iterative fashion

Table 1 Comparison of second virial coefficients

Temperature (°C)	Stockmayer*	Model CI†	BNS‡	ST2†	PE§	Experimental ($cm^3\ mol^{-1}$) Refs 34	56	33
100	−450	−827	−466	−1,023	−507	−450	−460	−507
200	−205	−289	−190	−355	−231	−197	−210	−214
300	−122	−141	−107	−177	−125	−112	−115	−113
400	−80	−78	−65	−104	−78	−72	−72	−72

*Ref. 32. †Ref. 57. ‡Ref. 12.

§The present model uses an isolated dipole moment of 1.855 D (ref. 19), absolute (not derived[23]) quadrupole moments ($Q_{xx} = -7.482$, $Q_{yy} = -4.180$, $Q_{zz} = -5.939$ D Å, axes as defined by Glaeser and Coulson[26]) from quantum mechanical calculations[24] and an isotropic polarisability ($\alpha_{xx} = \alpha_{yy} = \alpha_{zz} = 1.444$ Å^3) obtained from extrapolated[25] refractive index data. The Lennard–Jones parameters ($\sigma = 3.024$ Å, $\varepsilon = 0.764$ kcal mol^{-1}) were chosen to fit the equilibrium dimer[27] properties.

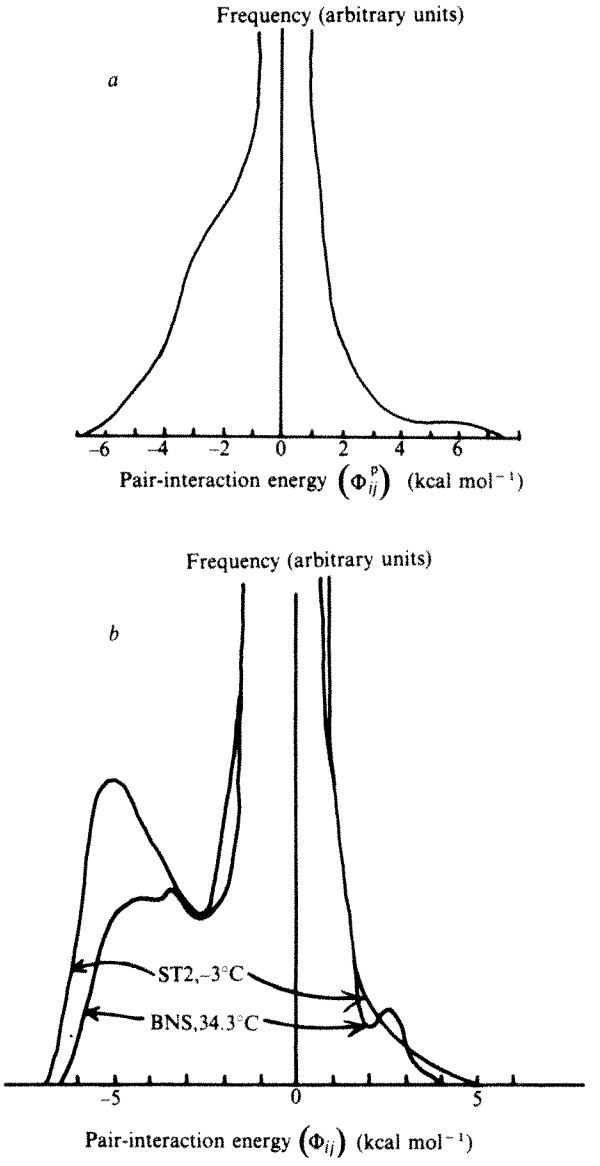


Fig. 2 The population distributions of interaction energies between all pairs of molecules in simulated configurations obtained using: *a*, the polarisable electropole: and *b*, four-point charge models. The four-point charge data (BNS[6] at 34.3 °C, ST2 (ref. 11) at −3 °C) show a definite low energy peak or shoulder at −3 to −6 kcal mol^{-1} which has been identified with 'hydrogen-bonded' pairs. In contrast the PE model indicates merely a broadening consistent with a picture of continuously-varying hydrogen bond energies rather than one in which bonds are discretely formed or broken.

(essentially a self-consistent field approach) and 2–5 iterations are typically necessary to achieve a constancy of better than 1% in the final charge distributions. Once this is completed, the energy is calculated by summing the pair interactions of electric fields with the resulting instantaneous dipole moments and field gradients with quadrupoles. The relevant equations are given elsewhere[42].

The model has the following advantages: (1) The non-pair-additivity calculations are exact. No effective averaging is used. Consequently this potential can be used for gas, liquid and crystal calculations as well as interfacial and mixed aqueous systems. (2) No adjustable electrical parameters are used, in contrast to other models[7,9,11,12,29] where parameters are adjusted to one or two sets of data. (3) Artificial devices such as switching functions[11] are not necessary. (4) Problems of generalising the switching functions to polarisable water molecules do not exist; electrostatic polarisation catastrophes are not experienced in practice except in cases of pathological configurations. (5) The properties of the isolated water molecule are by definition correct. In particular the quadrupole components, in contrast to four-point-charge models, are correct in both sign and magnitude.

Tests of the model

Before using any potential in expensive liquid-phase simulations it is only sensible to test it against known properties which are easily quantified[13,30]. If such tests give poor agreement with experiment, then the potential is clearly inadequate. One cannot overstress the need to be critical at this stage. Thus, we have preferred to gain confidence in the predictive ability of a potential function over a wide range of densities before testing it on expensive liquid, solution and biological interface calculations. The following is a selection of the tests made so far.

The second virial coefficient of steam $B(T)$: this two-molecule property, exploring the interactions of an isolated pair of molecules, can be written for a temperature T as

$$B(T) = -\frac{1}{16\pi^2}\int (\exp - \phi_{ij}/kT - 1)\,d\tau$$

Here, ϕ_{ij} is the interaction energy of the pair, and k the Boltzman's constant. The integration, ideally performed over all possible configurations, raises sampling problems which are discussed elsewhere[31]. Table 1 shows $B(T)$ for several potentials. The BNS and Stockmayer potentials were obtained by fitting adjustable parameters to $B(T)$ whereas the ST2, CI and our PE model values are predictions made without such adjustments. Of the latter set, only the PE model gives a satisfactory fit, especially at low temperature.

The equilibrium water dimer: the polarisable electropole (PE) predictions are in excellent agreement with the equilibrium configuration and force constants from both quantum mechanical calculations[7,17,18,28,36] and experimental measurements[27] (see Table 2).

Small water clusters: as agreement between different quantum mechanical calculations is poor, we discuss only the relative stabilities of the different cluster geometries, and the overall trends in their respective non-pair-additivity energies rather than the less reliable absolute values. Referring to Table 3, the most stable arrangements are the sequential (OH—OH—OH—) form with a distinct preference for the more closed chain varieties, and (except for the trimer) for cyclic clusters over chains. The PE model reproduces the same relative trends in equilibrium geometry and non-pair-additivity. The hydrogen-bond energy increases from −6.09 to −10.35 kcal mol^{-1} as the cluster chain grows from two to five molecules; the enhancement of some 70% is more than a mere perturbation. Water is clearly cooperative, and the PE model reproduces structural and energetic consequences well within the variations found between different quantum mechanical calculations.

Ices: theoretical estimates of the average dipole moment $\bar{\mu}$ in ice Ih suggest a value of 2.6 D (ref. 37)—an enhancement of 40% over the isolated molecule value—while a recent experiment proposes values between 2.45 and 3.0 D (ref. 20). The PE model predicts an average value of 2.88 D, with a half-width spread of about 0.3 D. (The PE model tests were performed on an enlarged unit cell of 432 ice molecules using normal periodic boundary conditions and a cut-off distance of 11 Å. Canonical Pauling arrangements for hydrogen positions were obtained using a disordering 'loop generation' technique described elsewhere[11,22].)

Compared to the experimental value of −11.3 kcal mol^{-1} for the internal energy of ice Ih (refs 38, 39), the PE model gives a value of −11.8 kcal mol^{-1}, of which −17.1 kcal mol^{-1} comes from electrostatic contributions. Although this agreement is encouraging, we regard it as largely coincidental in view of the unoptimised repulsive core parameters. The model is also consistent with the non-existence of a ferro-electric phase of ice

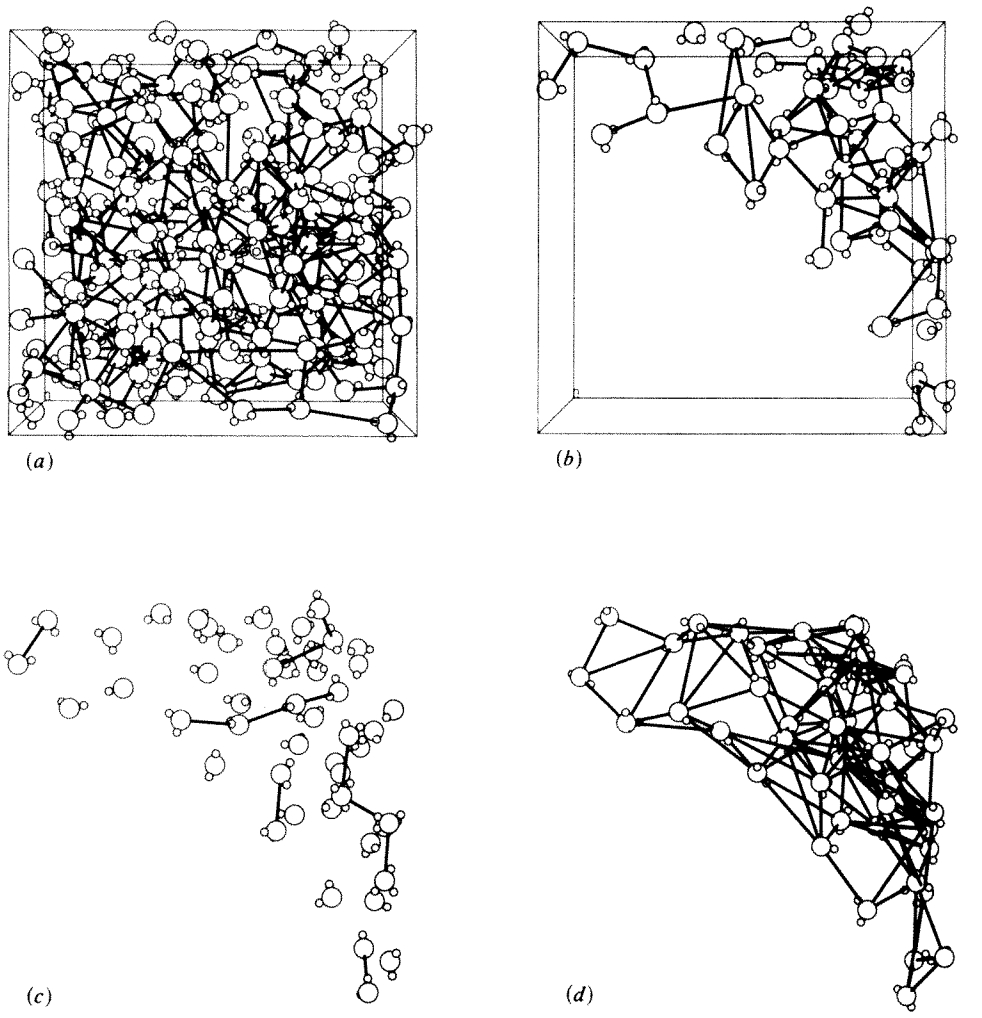


Fig. 3 Computer-drawn models of PE-simulated bulk water (*a*) and a cluster of 50 water molecules (*b–d*). The intermolecular links are notional hydrogen bonds chosen using an energy interaction criterion (cutoff at -2.0 kcal mol^{-1}, using polarised electropole pair-interactions) to give an average coordination of 4.0 in the bulk. The 50 molecule cluster was chosen from a bulk configuration (b-bulk molecules not shown), then removed from the bulk environment (*c*), and then allowed to move freely under the Monte Carlo process (*d*). The initial isolation from the bulk drastically changes the bonding patterns from that of *b* to *c*, strikingly demonstrating the effects of cooperativity at an interface. Pair-additive models would be unable to distinguish between the bonding of *b* and *c*.

Ih (ref. 40), in which the hydrogen positions would be ordered. Such an ordered phase would have a predicted potential energy marginally higher (0.4 kcal mol^{-1}) than the disordered phase. Although the difference is small ($\sim kT$), this alliance of increases in both entropy and binding energy is unusual. The high stability of the high pressure-hydrogen ordered ice VIII is also shown[41] by the PE model to be almost wholly due to dipole–quadrupole interactions, underlining again the need for the correct sign of the quadrupole moment.

Liquid water

We have begun to examine some consequences of non-pair-additivity for the liquid phase of 'PE water'. We stress the preliminary nature of these results, in particular the short chain length so far generated (see legend, Fig. 1a), and that the model is being refined. Nevertheless, some of the results make very interesting comparisons with those from standard pair-additive potentials.

The reconstructed instantaneous configuration shown in Fig. 3*a* is consistent with a picture of the liquid as a non-crystalline disordered network. Closer examination, however, shows considerably lower tetrahedrality of local coordination than found in ST2, BNS or Rowlinson-water[5,6,11,44]. Although surprising at first, this result is consistent with both the degree of tetrahedrality indicated in crystal hydrate studies[45] and the less structured picture of water given by recent neutron diffraction data[14,15] (Fig. 1*b*).

Figure 1 compares radial distribution functions of PE water with experimental X-ray[46] and neutron[14,15] results. There are evident deficiencies in the PE results (as there are deficiencies with pair-additive calculations[8]) which suggest, among other things, an inadequate treatment of the short-range repulsion; the improved agreement with the 50 °C curve suggests that our notional temperature is too low for the potential (repulsive part) used. The comparison with very recent neutron data is particularly interesting (Fig. 1*b*). Whereas ST2 and CF (ref. 51) both predict too much close-range structure (a consequence of over-tetrahedrality), the PE results are much more consistent with experiment. This less-structured picture is also borne out by studies[22] of the distribution of effective pair interaction energies as seen in Fig. 2*a*. The lack of definite bimodal distribution on the negative energy side should be compared with the definite maximum observed for ST2 and BNS water[46] (Fig. 2*b*) which was used to support the idea of a well-defined hydrogen bond. In contrast, the PE model is consistent with a picture of a continuously varying hydrogen-bond energy rather than formation (or breaking) of discrete hydrogen bonds. This suggests that it may be dangerous to identify hydrogen bonds from the purely geometrical considerations of distances and angles. In some cases, a second-nearest neighbour geometrically is found to be a first-nearest neighbour energetically and vice versa.

Table 2 Water dimer predictions

Model	DBP*	HMS†	D‡	PKC§	CI‖	PE¶	Experiment[27]
Energy minimum (kcal mol^{-1})	−6.09	−4.72	−4.83	−0.460	−5.87	−5.00	
θ (deg)	122	140	140–145	135–150	143	128	122°±10
γ (deg)	51	53	52	52	48	57	51°±10
r (Å)	2.73	3.00	3.00	3.00	2.98	3.0	2.98±0.04

*Refs 18, 36 predictions (quantum mechanical). †Ref. 17. ‡Ref. 28. §Ref. 43. ‖Ref. 7. ¶Refs 21, 42. The angles θ, γ refer to those between the $\widehat{HOH}$ bisector and the O–O separation r.

Table 3 Water cluster energies and non-pair additives (kcal mol^{-1})

Water cluster	Quantum mechanical	Polarisable electropole (PE)
DBP-trimers		
Double donor	−9.33 (+1.87)	−13.58 (+0.24)
Double acceptor	−10.14 (+2.05)	−13.66 (+0.55)
Sequential	−14.66 (−2.06)	−16.77 (−0.83)
Polar-cyclic	−16.83	+10.51 (−1.74)
DBP-tetramers		
Open	−23.45	−26.02 (−1.8)
Closed	−24.55	−27.25 (−2.2)
Polar-cyclic	−37.91	−32.62 (−7.55)
DBP-pentamers		
Semi-open		−36.25 (−3.12)
Closed	−41.39	−43.70 (−5.24)
Polar-cyclic	−53.15	−73.61 (−16.75)
DBP-hexamer		
Polar-cyclic	−64.53	−93.67 (−23.66)

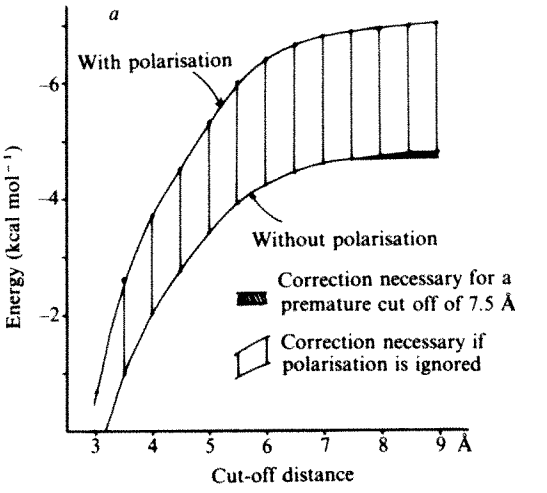
Non-pair additives are given in parentheses. The DBP-cluster configurations and the quantum-mechanical values are those given by Del Bene and Pople[18,36]. The PE values contain just the electrostatic portion—the Lennard-Jones contribution is irrelevant to non-additivity.

The distribution of instantaneous dipole moments yields an average (enhanced) value of 2.50 D with a half-width spread of about 0.7 D. The relevance of cooperativity to dielectric, and hence solution effects, is strongly underlined. The internal energy is −6.8 kcal mol^{-1}, of which −9.0 kcal mol^{-1} is electrostatic. The comparison with experiment[38] (−9.9 kcal mol^{-1}) is not good, but the above discussions of notional temperature and the sensitivity to the repulsive core should be borne in mind.

The significance of non-pair additivity at a free water interface is underlined by a Monte Carlo calculation in which a droplet of 50 water molecule centres (chosen with a large surface area) was instantaneously removed from a bulk simulated water configuration. The evolution of the removed cluster is shown in Fig. 3*b–d*. Note that the initial destruction of hydrogen-bonding interactions depends solely on removal of the remaining aqueous environment, and the rapid re-establishment of a new hydrogen-bonding scheme as the droplet adjusts to its isolation. Pair-additive potentials would be unable to differentiate between the bonding in the first two states (Fig. 3*b*, *c*). Hence we stress the inadequacy of using pair-additive potentials in looking at any aqueous interface, such as protein–water interactions[47,48].

The effects of truncating the energy calculation at a short distance, normally 7–9 Å, have been widely discussed for dipolar fluids[49,50]. The concept of a reaction field, whereby the back-reaction of the forces from the fluid outside the cut-off radius are considered, has been proposed[52,53] as a possible way round the problem. However, all these discussions have been within the framework of pair-additive potentials. When non-pair-additive effects are considered, there are significant changes in the nature of the problem.

Figure 4*a* shows the correction necessary to the energy of the 'water' assembly using different cut-offs, with and without polarisability. Clearly a premature truncation gives rise to a far



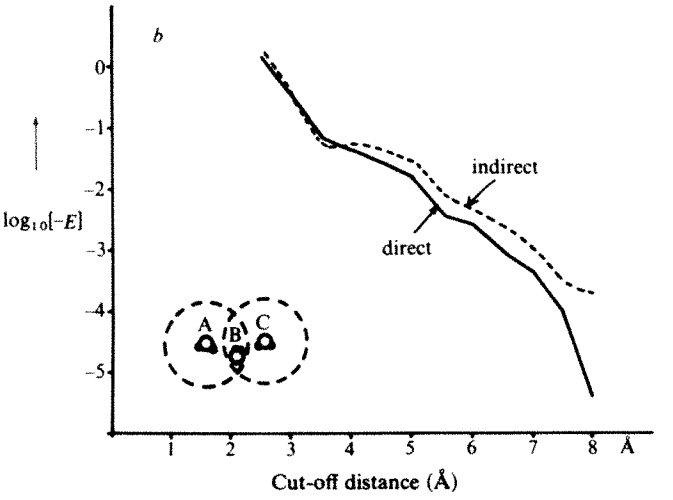


Fig. 4 *a*, The total energy of simulated PE-water as a function of the cut-off distance, with and without polarisation. The finely shaded portion represents the errors arising from using a cut-off distance of 7.5 Å (a common value), whereas the coarser shading indicates the errors arising from ignoring the cooperative effects of polarisation. Ignoring polarisation is clearly the dominant error. *b*, The variation with separation (r) of direct (——) and indirect (- - - -) interaction energies (E) between pairs of PE-water molecules in a bulk simulation. The indirect interactions arise from mutual polarisations through intervening molecules as illustrated by the three-molecule motif; the two molecules A and C (possessing cut-off spheres indicated by the larger circles) are separated so that no direct interaction exists between them, but a third molecule B common to both spheres provides, via mutual polarisation, an indirect link between A and C. Note that the indirect (A–C type) interactions overhaul the direct (A–B, B–C type) interactions for separations above 4 Å. This probing of long-range effects cannot be realised using pair-additive potentials.

smaller error in energy than does the neglect of the non-pair-additivity. A more subtle—and perhaps more dramatic—point is that within the framework of a non-pair-additive potential, we do in fact probe extensively those interactions beyond the cut-off radius via intermediate (polarised) molecules.

Figure 4*b* illustrates the point with three water molecules, A, B, C, placed so that A and C lie outside each other's formal cut-off distance. However, although A and C are considered to be unable to interact directly, the polarisation effects via molecule B provide an indirect link. We now define an indirect AC interaction as the difference between the potential energy of A with and without C present: clearly, only non-additive models will give non-zero values for this indirect contribution. Figure 4*b* compares the direct and indirect interactions as a function of the cut-off radius, and shows in our bulk water calculation that the indirect interactions are generally of greater consequence than direct interactions, even at the larger cut-offs.

Conclusions

It is necessary to abandon the use of pair potentials for aqueous systems. Although a parametrised effective pair potential reproduces 'homogeneous' properties such as the internal energy, we would have little confidence in its ability to predict properties depending on local effects, for example, the dielectric constant which depends significantly on the enhanced dipole moment. For interfaces, the removal of even a small part of the homogeneous fluid, for example by the presence of a solute molecule or a protein surface, will upset the indirect interactions severely (compare Fig. 3). We might draw a comparison with liquid metals, where we can devise an effective pair potential for a given density; change the density, and we change the effective potential. A bulk-derived potential would not be used for a liquid metal surface or for gas. Water should be thought of in a similar way. Interface calculations, be they simple solutions or protein crystals, based on pair-additive potentials would seem very premature.

Our approach to handling cooperative effects is probably only one of several which could be useful[54,55] and is clearly capable of further improvement. We would urge more effort be made towards developing alternative approaches, rather than continuing with applying known inadequate potentials[13,15] to more complex systems.

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Subduction, the geoid, and lower mantle convection

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Geoid models show that net uncompensated masses in subducting lithosphere are less than thermal models predict, and do not require elevation of mantle phase changes in the slabs. The largest deviations from hydrostatic equilibrium ignore plate boundaries and may be ascribed to lower mantle convection uncoupled to plate motions.

THE EARTH'S gravity field gives the most direct evidence on the distribution of mass in its interior. In particular, areas of anomalous density associated with thermal convection—cold subducting slabs, mantle upwellings, deflection of internal density boundaries—should be reflected in the global gravity potential. Some surface tectonic features have characteristic large scale gravity anomalies[1,2], especially the oceanic subduction zones with their associated geoid (Fig. 1) and gravity

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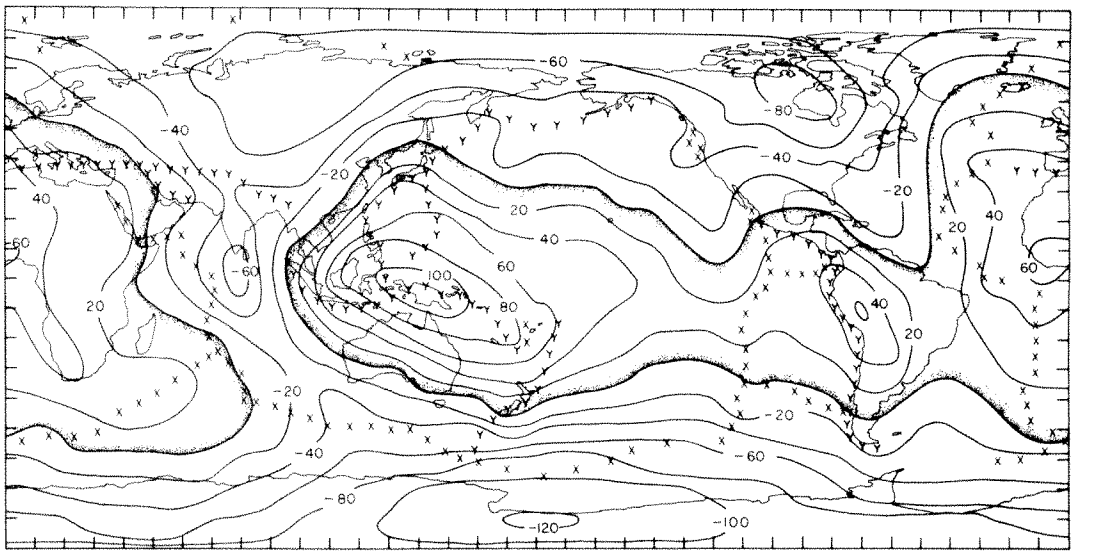


Fig. 1 Contours of the GEM8 geoid relative to a hydrostatic figure of flattening 1/299.75. In all the figures: solid contours are at 20-m intervals; dashed contours at intermediate 10-m intervals; uphill side of the zero contour stippled. Geoids were synthesised from coefficients of degree 2–20. Y, point on subduction zone; X, point on spreading centre. Cylindrical equidistant projection.

highs. However, most of the observed variation in the gravity field is not closely related to plate boundaries. Here forward modelling is used to match the observed field, thereby placing limits on the size and depth of some of the anomalous regions. Most previous quantitative interpretations of global gravity[3–9] have used direct or statistical inversion of the gravity potential.

The geoid

It proved advantageous to work with the geoid rather than with global gravity anomalies, which amplify the higher degrees of the spherical harmonic expansion of the potential. In geopotential models those coefficients are not as well determined as the lower degrees. Thus the geoid, which weights the harmonics evenly, is less subject to errors of measurement. The geoid also 'sees' deeper than the gravity field, because the effect of a mass on the geoid decreases directly with depth, while the gravity effect decreases with the square of the depth. Because the geoid is not as sensitive to the exact depth of perturbing bodies, their masses can be estimated more reliably without knowing the details of their vertical distribution.

All departures from hydrostatic equilibrium within the Earth must be supported by the strength of rocks or by viscous stresses, so the appropriate geoidal reference surface for tectonic purposes is the hydrostatic rotational figure of the Earth. Figure 1 shows the elevation of the nonhydrostatic geoid for GEM8[10]. The shortest wavelength represented is ~1,000 km.

Subduction zone model

It seems to be accepted that excess (uncompensated) mass of the subducting slabs is important in causing oceanic slabs to move (see ref. 11 and refs therein). Watts and Talwani[12] inferred from gravity models of outer rises that the cold slab was somehow regionally compensated. However, Grow and Bowin[13] have criticised their method and conclusion. Uncompensated slabs are used here since large averaging depth causes the geoid to respond most strongly to net excess or deficit of mass.

The starting point was the thermal slab model of McKenzie[14] as modified for finite slab length by Molnar and Gray[15]. The slab's down-dip length was taken as length of the seismic zone[16], except for Patagonia and the continental collision zones, where slabs to 200 km were assumed. Approximating points (Figs 1, 2) were then assigned masses determined by the local subduction rate from a global relative motion model[17] and the area of slab they represent. Behind-arc spreading was not taken into account. Depths of the point masses are shown in Table 1. Spherical harmonic coefficients of the point mass potentials[18] were then calculated and summed.

The simple thermal models markedly overestimate the net excess mass localised in most subducting slabs, turning positive anomalies into negative ones. An empirical subduction zone model (Fig. 2) was derived by independently adjusting the amount of excess mass for each subduction zone, usually downwards (Table 1), until the residual geoid (Fig. 3) showed as

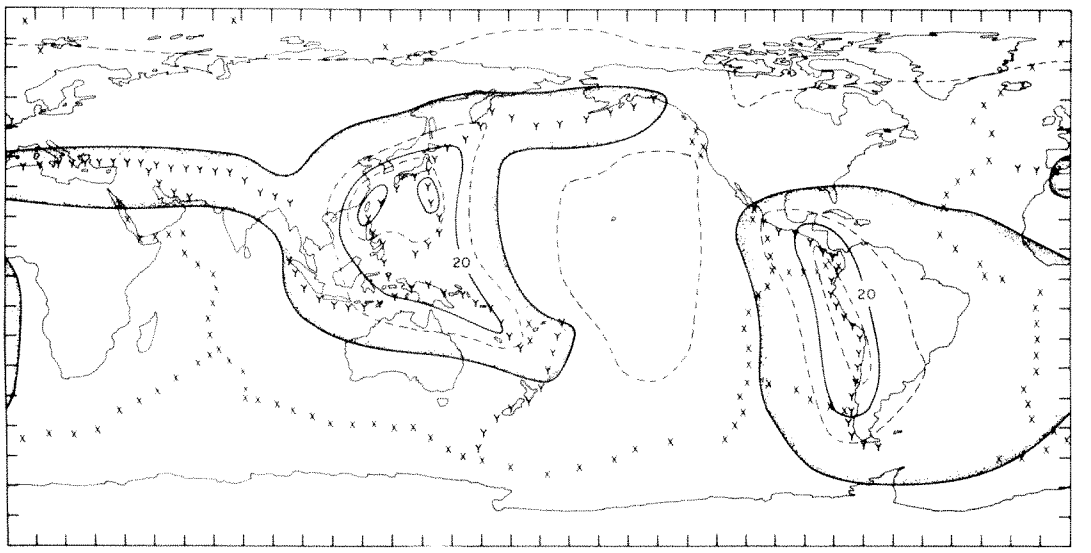


Fig. 2 Model of geoidal undulations due to excess uncompensated masses localised in cold subducting slabs (Table 1). Note the broad, low amplitude high in the South Atlantic. This is a 'farside' effect caused by displacement of the centre of mass towards the subduction zones in the Pacific. Centre of mass offset is 16 m towards 23° N, 165° E.

Table 1 Subduction zone (cold slab) and deep source models

Feature	Depth (km)	Length (km)	Excess mass (10^{15} kg km^{-1})	Cold slab theory (10^{15} kg km^{-1})*
Subduction zones				
Middle America	200	2,864	1.10	1.03
Peru–Chile	325	6,383	1.08	3.60
Patagonia	150	1,601	0.26	0.18
Tonga–Kermadec	350	3,708	0.41	2.37
New Hebrides–New Guinea	325	5,129	1.24	1.38
Java	350	5,384	0.37	2.35
Mariana–Bonin	375	3,498	1.33	2.29
Philippine	325	2,989	0.77	1.49
Ryukyu	325	2,215	1.62	0.89
Honshu	325	966	1.11	4.25
Kuriles	300	2,083	0.64	2.64
Aleutians	250	4,002	0.70	1.40
Mediterranean	250	5,608	0.24	0.20
Zagros	150	2,256	0.22	0.45
Himalaya	150	5,196	0.27	0.36
Deep sources				
Pacific	2,000–2,900	16,863	15.8	
Africa	2,000–2,900	18,651	11.9	
India–Antarctica	1,000–1,200	17,300	−4.18	
North America	1,000	6,111	−4.30	
Siberia	1,000	1,671	−4.29	
Arabia	1,200	(only 1 point)	8.36×10^{18} kg	

* Calculated from slab model of Molnar and Gray[15] using down-dip length of seismic zone from Isacks and Molnar[16], lithospheric thickness 100 km, $T_1 = 1{,}200$ K, $\rho = 3{,}300$ kg m^{-1}, $c_p = 1{,}050$ J kg^{-1} K^{-1}, $\alpha = 3\times10^{-5}$ K^{-1}, $\kappa = 3.77$ W m^{-1} K^{-1}, convergence rates from ref. 17.

little correlation with the position of the subduction zones as possible.

The subduction zone model (Fig. 2) has positive masses only but negative anomalies in some places, and positive values in places where there are no anamolous masses. The omission of zero and first degree terms from the gravity field means that a positive excess mass will have a positive geoid anomaly directly over it, negative values at greater distance, and a gentle positive on the opposite side of the Earth. This 'farside' effect is caused by displacement of the centre of mass towards the perturbing body.

There may be several reasons that the final model required less mass than theory suggested. The thermal model may not account for all important processes of heat transfer and generation. Because the topography of the oceanic trenches is probably not locally compensated, a trench can contribute up to 0.5×10^{15} kg km^{-1} of mass deficit to counteract the slab's contribution. There may also be local phase boundary deflections caused by convection, and thermal anomalies beneath back-arc basins that partially compensate the cold slab. Elevation of the olivine–spinel phase transition within the cold slab[19,20], increasing its excess mass, would worsen misfit of the thermal model. Sung and Burns[21] suggest that sluggish kinetics will delay transitions in the cold heart of the slab to greater depths, reducing or reversing the effect of the phase change.

A modest amount of uncompensated slab was permissible for continental collision zones of the Himalaya, Zagros and Mediterranean areas. This is surprising considering the importance of the Himalaya in development of isostatic theory, but there is some support from surface gravity studies[22].

The subduction zone model does not remove all geoidal highs located near the trenches, leaving broad positive anomalies on the continental side of the East Asian and Peru–Chile trenches (Fig. 3). These may reflect the pressure gradient necessary to drive asthenospheric counterflow from trenches to ridges. Global models of the flow[23–25] suggest that geoidal elevation should be roughly symmetrical about most convergence zones, but the residual highs are not. However, cooling of the oceanic lithosphere can reduce the elevation on the ocean side of the trench[26].

Another possibility has been pointed out by F. M. Richter (personal communication). Mantle cooled by the subducting

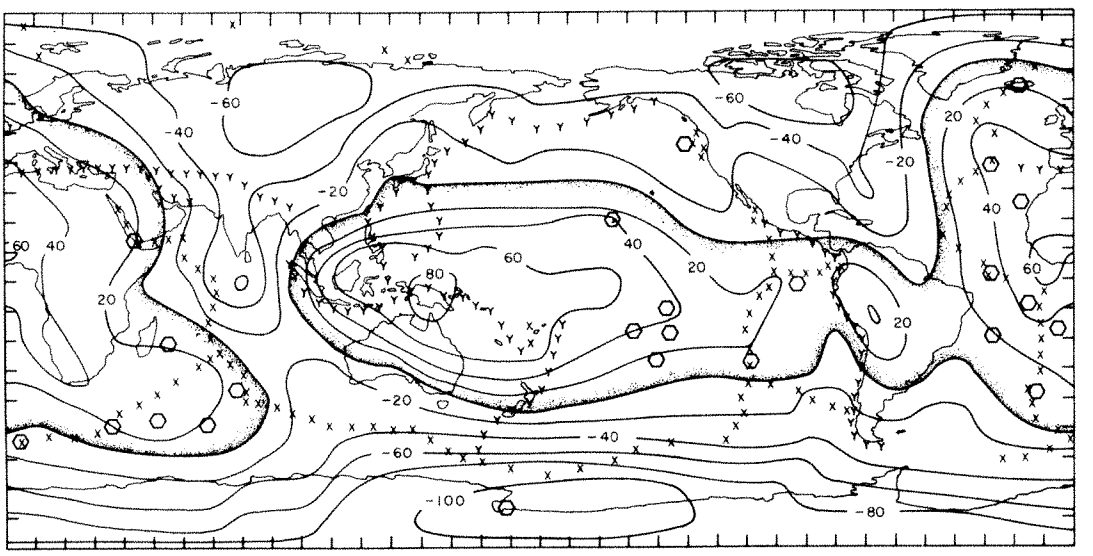


Fig. 3 Residual after subtraction of subduction zone model (Fig. 2) from observed geoid (Fig. 1). Large amplitude and wavelength anomalies remain. ⬡, The location of selected hotspots, mostly oceanic. They cluster strongly about the positive anomalies, mostly near the +20 to +40 m contour.

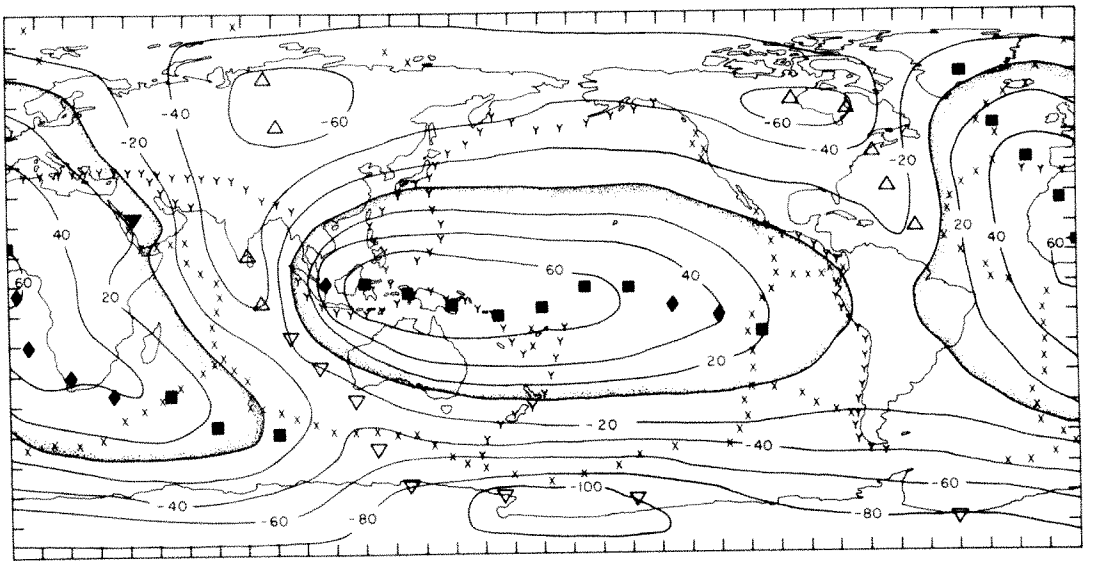


Fig. 4 Model of deep uncompensated point masses designed to approximate residual of Fig. 3. Depths: △, 1,000 km; ▽, 1,200 km; ◇, ◆, 2,000 km; ■, □, 2,900 km. Open symbols, negative point masses; filled symbols, positive point masses. Note the continuity of the anomalies and that the negative masses are all shallow. Centre of mass offset 64 m towards 6° N, 64° E.

slab cannot be removed from near the slab faster than conduction (very slow) or convection permits. This could leave a broad, fairly shallow dense area behind the subduction zone. Note that the residual highs are located where the subduction zones used to be, because all of these trenches move oceanwards in the hotspot frame of reference[27].

Large residual anomalies

The most remarkable features in Fig. 3 are three enormous 60–80-m amplitude geoidal anomalies. There are two positive features—one in the Pacific, stretching from Sumatra to the Galapagos, and the other from south-east of Africa to Greenland—and one discontinuous negative anomaly from Antarctica through India and Siberia to the North Atlantic. Clearly most variation of the geoid from hydrostatic equilibrium is not due to subduction zones.

These three large geoidal anomalies share several characteristics that need explanations. (1) Great size: each of the anomalies is at least 18,000 km in length, and the positive anomalies are more than 6,000 km wide, the negative is about 4,500 km wide. (2) Large amplitudes: all have amplitudes greater than 40 m for much of their length. (3) Lack of correlation to surface tectonic features: all three anomalies cross several plate boundaries with little apparent disturbance. There is no correspondence to type or age of lithosphere. (4) Strong correlation to location of oceanic hotspots: of the 24 selected hotspots marked on Fig. 3, all but three lie within the positive anomalies.

Deep source model

To find maximum source depth, I have constructed a point mass model for the large residual anomalies (Fig. 4). The point sources for the positive anomalies could be placed quite deep, at 2,000 or 2,900 km (core–mantle boundary). This depth is a maximum, because a broad, smooth anomaly can also come from a more gradual density fluctuation at shallower depth. The sources of the negative anomaly could be no deeper than 1,200 km, however. The estimate of excess mass for the features (Table 1) is a close upper limit and not very sensitive to depth of the source mass distribution. The positive anomalies, as well as being broader and possibly deeper in origin, require much more excess mass than the negative one.

This model for the non-hydrostatic part of the Earth's shape includes the excess equatorial bulge. The largest anomaly, the equatorial Pacific high, determines the maximum non-hydrostatic moment of inertia of the Earth, and therefore the position of the rotational axis[28]. Should the African high grow larger than the Pacific high, the whole Earth would shift relative to the axis—true polar wander, with Greenland moving to the Equator!

The observed geoid with the deep source model subtracted is shown in Fig. 5. Subduction-related features are most prominent (compare Fig. 2). There are other anomalies of note: positive anomalies of 2–8 m height over the mid-ocean ridges (see ref. 29); and a 10-m high in mid-Pacific west of the Hawaiian Islands[30]. The broad low in the eastern Pacific may be related to asthenospheric counterflow, as discussed above.

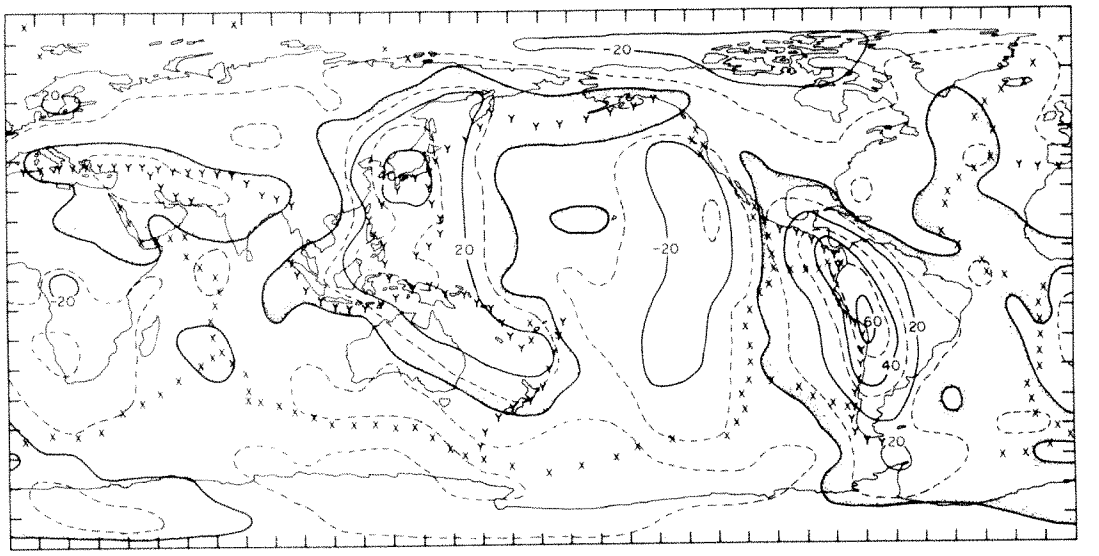


Fig. 5 Observed geoid with deep mass model of Fig. 4 removed. Compare with Fig. 2.

Lower mantle convection?

What, then, is the origin of the large anomalies (Fig. 3) that dominate the non-hydrostatic part of the Earth's geoid? Rocks are not strong enough to support these features, so convective processes must be responsible[3,5,31]. The lack of correlation of the large anomalies to plate boundaries and motions allows a more specific interpretation of where the convection is located: it must be below the flow regime associated with plate tectonics.

Whole mantle convection closely coupled to surface plate motions has been invoked lately[25,32-34], but is clearly not permissible if the large geoidal anomalies unambiguously reflect the location of lower mantle convection cells. Such a geometry could not satisfy mass balance requirements for plate motions[24] and there is no particular relationship to plate motions or interplate stresses[11]. For example, convection associated with the Pacific high should either extend or compress the Pacific plate in a north–south direction, neither of which is observed[11]. Thus the residual geoidal anomalies are direct evidence favouring the model advanced by McKenzie and Weiss[35] of two layer convection with upper and lower mantle circulating separately.

It is not obvious whether the positive anomalies would mark upwellings or downwellings in the lower mantle convection. Analytical[36] and numerical[37] models of mantle convection suggest that deflections of density boundaries will dominate the gravity field in many cases, leading to positive anomalies over upwellings. The tendency of hotspots to occur over geoidal highs may indicate hotter than normal material below. The hotspots themselves imply some interchange of material between lower and upper mantle, if they do originate within a lower convective system. Global heat flow patterns[38] show little correlation with the large geoidal anomalies. Seismic travel times under the North Atlantic[39] resemble the geoidal patterns there, slow travel times corresponding to geoidal lows. Globally, deep travel time anomalies[40] also show some correlation with geoid anomalies in the low degree harmonics. A great deal more work needs to be done on the intriguing possibility that the Earth's gravity field reveals convective patterns hidden at depth.

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Specificity of the antibody response of rabbits to a self-antigen

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The demonstration that the antigenic determinants on rabbit cytochrome c that elicit antibodies in rabbits occur in regions of variability among mammalian cytochromes c suggests the existence of an evolutionary process which eliminates genetic specificities for self-determinants.

DESPITE the considerable attention which has been given to the antigenic properties of proteins, we still do not know why certain parts of a protein antigen elicit antibody responses while other regions remain immunologically silent. As the B-cell specificity repertoire that is expressed is limited[1], it follows that not every molecular substructure of an antigen would have a corresponding B cell reactive to it. But what restraints act to limit the B-cell specificity repertoire?

One theory is that, because of the phenomenon of immunological tolerance, antibodies may not be elicited against those regions of an antigen which share structural or chemical properties with self-molecules of the host. Indeed, studies on the antigenicity of cytochrome *c* indicate that the regions of amino acid sequence difference between the cytochrome *c* immunogen and the corresponding host's protein are antigenic[2–5]. For example, one of the six residue differences between horse and rabbit cytochromes *c* is residue 60, where horse cytochrome *c* has lysine and the rabbit protein has glycine. These two cytochromes *c* have 18 other lysyl residues in common. When rabbits are immunised against horse cytochrome *c*, the antibodies directed against the region of lysine 60 comprise 20–40% of the total antibody and the other lysyl residues do not elicit antibody responses[4,5]. It is difficult to explain this observation other than by tolerance. The concept of a particular chemistry inducing antigenicity must be excluded in this instance as only one of 19 lysine residues is antigenic.

Although immunological self-tolerance seems to be a dominant factor affecting the specificity of the immune response to cytochrome *c*, it was early observed that not only did antibodies elicited against a heterologous cytochrome *c* bind the rabbit protein, but also when polymerised with glutaraldehyde[6]

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or covalently coupled to bovine γ-globulin[7], rabbit cytochrome *c* itself was immunogenic in rabbits. The finding that antibodies elicited against a foreign cytochrome *c* cross-reacted with rabbit cytochrome *c* is attributed to the more stringent requirements for stimulating a B cell than for binding of an antigen to the cell receptor (antibody)[8,9]. Thus, the observation is not inconsistent with the existence of a state of tolerance in rabbits to rabbit cytochrome *c*. However, the finding that rabbit cytochrome *c*, when in certain molecular forms, does initiate an antibody response is not as readily understood. The quantitatively lower response evoked by the self-antigen, as opposed to heterologous cytochromes *c*, indicates a degree of immunological tolerance to rabbit cytochrome *c* and it may be that modification by coupling to a carrier or by polymerisation may have enabled the protein to trigger a response that would normally not be allowed.

In any case, the ability to elicit an antibody response to a self-antigen indicates that immunological self-recognition is not the only mechanism allowing certain sites on an antigen to remain immunologically silent. We considered that by establishing which sites on rabbit cytochrome *c* elicit antibody responses in rabbits, we might elucidate factors other than lack of self-recognition that would influence the antigenicity of only certain regions of a molecule.

To obtain autoantibodies against rabbit cytochrome *c*, eight adult New Zealand white male rabbits were injected three times over 6 weeks with the glutaraldehyde-polymerised form of the protein (~9 mg total) emulsified in Freund's complete or incomplete adjuvants. The cytochromes *c* used in this study and the glutaraldehyde-polymerised rabbit cytochrome *c* were prepared as previously described[3]. Rabbit cytochrome *c* was chemically fragmented using cyanogen bromide and the peptides (residues 1–65, 66–80 and 81–104) were separated according to the procedure of Corradin and Harbury[10]. Other methods used, including antibody fluorescence quenching titration and modified Farr assay, have been reported elsewhere[3].

Characterisation of the antibody response

All eight rabbits immunised with rabbit cytochrome *c* polymers produced antibodies specific for the monomer, with serum titres between 115 and 230 μg antibody ml^{-1} as determined by immunoadsorption of the antibodies on rabbit cytochrome *c* coupled to CNBr-activated Sepharose 4B. The serum titres were 5–20% of the response observed against heterologous cytochromes *c* differing from the rabbit protein at 6–9 residues out of 104. Clearly, rabbits display a degree of 'tolerance' to their own cytochrome *c*[6].

The rabbit anti-rabbit cytochrome *c* antibodies adsorbed on the insolubilised antigen had an apparent molecular weight of 150,000 based on gel filtration studies on a calibrated column of Sephadex G-150, migrated electrophoretically as a single band

Table 1 Comparison of the amino acid sequences of mammalian cytochromes *c*

	3	3	4	4	5	6	6	8	8	9	10
Cytochrome *c*	3	5	4	7	8	0	2	8	9	2	3
Rabbit[12]	H	L	V	S	T	G	D	K	D	A	N
Mouse[13,14]	–	–	A	–	–	–	–	–	G	–	–
Kangaroo[15]	N	I	P	–	I	–	–	–	G	–	–
Guanaco[16]	–	–	–	–	–	–	E	–	G	–	–
Beef[17]	–	–	P	–	–	–	E	–	G	E	–
Horse[18]	–	–	P	T	–	K	E	–	T	E	–
Dog[19]	–	–	P	–	–	–	E	T	G	–	K

The single letter code for amino acids is used. Residue positions are given vertically, for example, $\frac{3}{3}$ referring to position 33. Only the residues that differ among the cytochromes *c* listed are shown. – Indicates that the residue is identical to that of the rabbit protein.

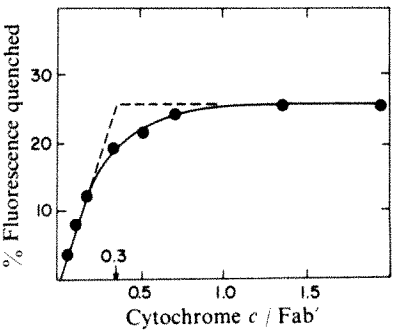


Fig. 1 Fluorescence quenching titration of rabbit anti-rabbit cytochrome *c* Fab' with rabbit cytochrome *c*. The antibodies were isolated from pooled antisera on insolubilised rabbit cytochrome *c*, digested with 2% pepsin for 8 h, then reduced with cysteine and alkylated with iodoacetamide.

in the γ region, and showed precipitation in agarose film with goat anti-rabbit γ-chain-specific but not μ-chain-specific antisera. Thus, the antibodies elicited in rabbits against their own protein were IgG. The stoichiometry of the antibody-binding reaction, as determined by fluorescence quenching assays between the Fab' fragments prepared from the antibodies and rabbit cytochrome *c*, was 3 : 1, indicating the presence of at least three separate antigenic determinants on rabbit cytochrome *c* (Fig. 1).

Modified Farr assays of rabbit anti-rabbit cytochrome *c* sera showed that the antibodies distinguished between rabbit and heterologous cytochromes *c* that differed at two to six amino acid residue positions (Fig. 2, Table 1). Thus, mouse cytochrome *c*, which varies from the rabbit protein at only two positions—residues 44 and 89—did not compete with ^{125}I-labelled rabbit cytochrome *c* for the antibody-binding sites as effectively as did rabbit cytochrome *c*. The dog protein displaced only 30% of the labelled rabbit cytochrome *c*, even though it differs from the rabbit protein at only five residue positions—44, 62, 88, 89 and 103. These results show that the rabbit anti-rabbit cytochrome *c* antibodies are directed against regions of the molecule where the rabbit and the other mammalian cytochromes *c* differ in amino acid sequence.

Identification of the sites of antibody binding

At least one antibody population bound in the carboxyl-terminal segment comprising residues 88–104. Indeed, dog cytochrome *c* was a less effective inhibitor in the modified Farr assay than was the beef protein, which differs from it only at residues 88, 92 and 103. Furthermore, the peptide consisting of residues 81–104 of rabbit cytochrome *c* inhibited the binding of approximately 20% of the labelled rabbit cytochrome *c* when present in a 20-fold molar excess over the latter (Fig. 3). The difference in binding between mouse and rabbit cytochromes *c* may indicate that the antigenic site is in the region of residue 89 as these two proteins differ only at that position and at residue 44. In any case, the data indicate that residue 44 or 89 or both are within antigenic determinants. In contrast to the 81–104 peptide, the 66–80 peptide failed to inhibit any binding of the antibodies to rabbit cytochrome *c*. However, the haem-containing 1–65 peptide displaced 80% of the labelled protein. Thus, the 1–65 and the 81–104 peptides contained the whole of the autoantigenic activity of rabbit cytochrome *c*. Further one of the antibodies binding to the 1–65 fragment was found to be directed against the region of residue 62. Thus, mouse and kangaroo cytochromes *c*, which, like the rabbit protein, carry aspartic acid at that position, were more effective inhibitors of rabbit cytochrome *c* than was guanaco cytochrome *c*, which has glutamic acid (Fig. 2). The only other position at which guanaco cytochrome *c* differs from the rabbit protein is 89, where the former

has a glycine and the latter aspartic acid. Both mouse and kangaroo cytochromes *c* are identical to the guanaco protein at position 89, so that the greater affinity of these two cytochromes *c* for the antibodies must be due to their greater similarity to the rabbit protein at position 62.

As the stoichiometry of the reaction between rabbit cytochrome *c* and its antibodies is three, in addition to the antibodies binding in the carboxyl-terminal segment and in the region of residue 62 there is at least one other antibody population. It probably binds to the amino-terminal segment of the protein represented by the 1–65 peptide, because as already noted, that peptide accounts for a large proportion of the autoantigenic activity of the molecule, and the 81–104 fragment contains another antigenic determinant accounting for the remainder of the activity.

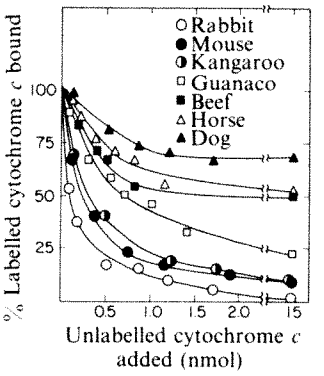


Fig. 2 Competitive binding assays between rabbit and various mammalian cytochromes *c* and antibodies elicited in rabbits against the glutaraldehyde-polymerised form of rabbit cytochrome *c*. Rabbit cytochrome *c* was iodinated with carrier-free ^{125}I by the method of Hunter and Greenwood[20]. The antiserum used was pooled from four rabbits and diluted 1:4 with 6.2 mM sodium borate–boric acid buffered at *p*H 8.6, containing 130 mM sodium chloride. Each reaction mixture contained 1.36 nmol ^{125}I-labelled rabbit cytochrome *c*, 0.20 ml diluted antiserum and amounts of unlabelled cytochrome *c* ranging from 0 to 15 nmol. Antibody–cytochrome *c* complexes were precipitated in 50% saturated ammonium sulphate and washed three times. The amount of radioactivity precipitated was corrected for nonspecific binding using normal rabbit serum. In the absence of inhibitor 0.038 nmol of ^{125}I-labelled rabbit cytochrome *c* were precipitated.

Do specificities for self-determinants tend to be eliminated during evolution?

From the above analysis, two of the three antigenic sites on the self-antigen rabbit cytochrome *c* have been localised to the regions around residue 62 and the carboxyl-terminal segment probably around residue 89. Those two sites correspond to regions on heterologous cytochromes *c* that are antigenic in rabbits. Indeed, the antigenic sites on guanaco and mouse cytochromes *c* were found to be centred around residues 62 and 89 and residues 44, 62 and 89, respectively[3], and those on horse cytochrome *c* were in the regions of residues 44, 60 and 89 or 92 (refs 4, 5). The area around residue 44 in rabbit cytochrome *c* may be the third autoantigenic site of that protein.

What property of these regions of cytochrome *c* makes them antigenic? It is unlikely that the particular amino acid side chains share an antigenic property, as each cytochrome *c* examined with respect to antigenic specificity has different amino acid residues in the immunodominant position and the antibodies elicited by each cytochrome *c* antigen are specific for its own side chains[2–5]. Possibly, the tertiary structure of the molecule in the antigenic regions is such that interactions with cells of the immune system occur more readily. This explanation also seems unlikely as the cytochrome *c* molecule is a tightly folded oblate spheroid and no externally located backbone sequence is particularly better situated than any other for increasing the probability of extramolecular interactions. In this regard, there are no sharp bends in cytochrome *c* as there are in myoglobin that could explain the antigenicity of certain sites on that molecule[11].

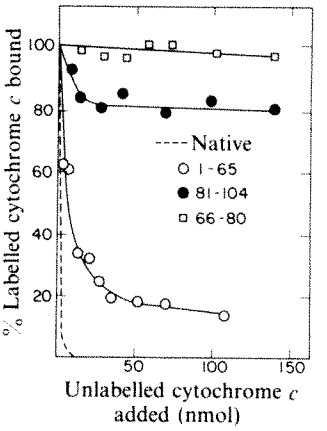


Fig. 3 Competitive binding assays between rabbit cytochrome *c* and cyanogen bromide-cleaved peptides of the protein and antibodies elicited in rabbits against rabbit cytochrome *c*. The assays were carried out as described in Fig. 2 legend.

Notwithstanding any unknown chemical features which of themselves might allow certain regions to be antigenic, it is particularly interesting that the antigenic sites on rabbit cytochrome *c* correspond to the areas on the molecule where other mammalian cytochromes *c* differ from it (Fig. 4) and in fact, a region in which all cytochromes *c* are identical (residues 66–80) was demonstrated not to be antigenic in rabbit cytochrome *c*.

A possible explanation for this is that only certain regions of a self-antigen elicit immune responsiveness because other specificities are absent from the repertoire (not carried in the germ line). Selective pressures against the maintenance of antibody specificities to self-antigens could act during evolution to eliminate specificities for self-antigenic determinants from the antibody repertoire. Because self-antigens would continue to vary structurally in the course of evolution, as selective pressures begin to effect the elimination of the corresponding antibody specificities, those specificities corresponding to the most variable regions of a self-antigen, namely, those most likely to have varied recently, are the least likely to have been eliminated. Selective pressures would be most effective against those antibodies specific for the evolutionarily conserved regions of a self-molecule because of the long exposure time required for evolutionary elimination.

Thus, if this interpretation is correct, antibodies elicited against a self-antigen should correspond to those regions of the molecule which have varied evolutionarily. That seems to be the

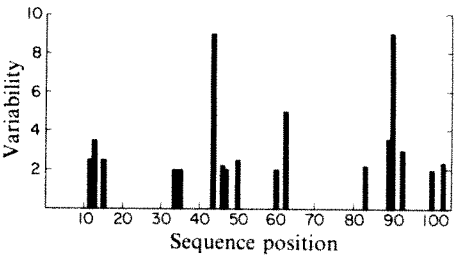


Fig. 4 Variability of mammalian cytochromes *c*. Variability is defined as the number of different amino acids at a given position divided by the frequency of the most common amino acid at that position[21]. Eighteen mammalian cytochrome *c* sequences were used for this[22,23]. The variabilities at residues 11, 12 and 15 are attributable to amino acid substitutions in the later evolving primate cytochromes *c* and thus, the region around residues 11, 12 and 15 would not be antigenic in rabbit cytochrome *c* by the mechanism discussed in the text.

case with the antibody response in rabbits against rabbit cytochrome *c*. One could, therefore, consider that there are at least two biological levels at which an individual is rendered tolerant to self-antigens—at the developmental level, clones reactive with self are aborted or tolerised; at the evolutionary level, genes corresponding to specificities against self tend to be eliminated. Although this is not the only possible interpretation, it would be consistent only with a germ-line or programmed somatic mutation theory of antibody diversity and cannot be reconciled with a theory of diversity involving somatic mutation alone.

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Complete nucleotide sequence of an influenza virus haemagglutinin gene from cloned DNA

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A synthetic fowl plague virus (FPV) haemagglutinin gene has been cloned in bacteria and the complete sequence of the RNA gene deduced. It is 1,742 nucleotides long and the mRNA codes for 563 amino acids in an uninterrupted sequence. The nature of some of the important domains in the haemagglutinin has been established, and their structure is discussed in relation to their function. Extensive amino acid sequence homologies exist between FPV and human influenza haemagglutinins.

FOWL PLAGUE VIRUS (FPV) like other influenza viruses is a negative-strand virus with a genome of eight separate[1–3] and unique RNA segments[3], two of which code for the surface antigens, haemagglutinin (gene *4*)[4–6] and neuraminidase. The haemagglutinin (HA) is responsible for attaching the virus to cell receptors[7] and for initiating infection[8,9]. It is firmly established that host antibodies are directed against the haemagglutinin[10,11], and that the virus can escape neutralisation by spontaneous mutation, resulting in amino acid changes in the haemagglutinin. These changes may be major and cause pandemics (antigenic shift) or relatively minor and cause epidemics (antigenic drift)[12]. The minor antigenic changes occur more frequently, but their precise nature and location remain unknown.

Due to the difficulty of RNA sequencing with a molecule the size of the HA gene, peptide sequencing was, until recently, the best method of studying antigenic variation of influenza viruses. Ward and Dopheide[13] and Waterfield and Skehel[14] have determined most of the peptide sequence of the mature HA of a Hong Kong strain (H3N2) and an Asian strain (H2N2), respectively. With the introduction of gene cloning and rapid DNA sequencing greater speed is obtained, and certain problems such as the difficulty of sequencing hydrophobic peptides are overcome. In addition, the gene sequence not only predicts the complete amino acid sequence of the mature protein, but will also indicate the sequence of any peptides that are discarded during its maturation and perhaps also if untranslated sequences (introns) interrupt the coding region of the gene.

Gene sequences can also provide information about the structure of genes not evident from the protein sequence, and a means of comparing different virus strains in greater detail than techniques such as hybridisation[15], gel electrophoretic mobility[16] and oligonucleotide mapping[17].

In this article we describe the determination of the complete nucleotide sequence of the FPV haemagglutinin gene using recombinant DNA techniques. The predicted sequence of the HA allows us to correlate some of the known functions with specific structures in the molecule and to compare the amino acid sequence with partial sequences of human influenza haemagglutinins[13,14].

Gene *4* sequences in plasmid pBR322–FPV$_{4-10}$

We chose to polyadenylate the eight virion RNA segments of unfractionated FPV RNA, and reverse transcribe the mixture with an oligo(dT) primer, since the resultant transcripts included substantial numbers of full length or almost full length copies of all eight genes[18].

Table 1 Use of codons in FPV gene *4*

Phe	UUU 13	Ser	UCU 4	Tyr	UAU 7	Cys	UGU 7
	UUC 13		UCC 4		UAC 8		UGC 9
Leu	UUA 6		UCA 13	*Ochre*	UAA 1	*Opal*	UGA 0
	UUG 9		UCG 1	*Amber*	UAG 0	Trp	UGG 8
Leu	CUU 9	Pro	CCU 3	His	CAU 7	Arg	CGU 1
	CUC 4		CCC 1		CAC 4		CGC 0
	CUA 4		CCA 8	Gln	CAA 14		CGA 4
	CUG 8		CCG 4		CAG 11		CGG 3
Ile	AUU 17	Thr	ACU 13	Asn	AAU 23	Ser	AGU 11
	AUC 8		ACC 12		AAC 14		AGC 8
	AUA 15		ACA 15	Lys	AAA 22	Arg	AGA 14
Met	AUG 11		ACG 1		AAG 7		AGG 8
Val	GUU 9	Ala	GCU 12	Asp	GAU 17	Gly	GGU 5
	GUC 6		GCC 3		GAC 9		GGC 10
	GUA 5		GCA 17	Glu	GAA 28		GGA 20
	GUG 10		GCG 1		GAG 10		GGG 15

Codons with CG are underlined. 20.0% of codons used end in C, 32.9% end in A, 19.0% end in G and 28.1% end in U.

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To clone individual genes, the S_1-nuclease-treated double-stranded FPV cDNA was ligated to *Hin*dIII cut plasmid pBR322 DNA[19] using the *Hin*dIII linker method[20] (see Fig. 1 legend). The mixture was then used to transform[21] *Escherichia coli* K12 HB101. Thirteen tetracycline-sensitive colonies were obtained most of which contained plasmids with small gene inserts as judged by agarose gel electrophoresis. One plasmid, named pBR322–FPV$_{4-10}$, contained a large insert of about 1,700 nucleotides (Fig. 1, lane c) and the plasmid DNA specifically hybridised to a ^{32}P-labelled gene *4* probe[23] (see Fig. 1 legend for details). 59.7% of gene *4* probe was hybridised compared with not more than 3.1% for probes derived from genes *1–3* and *5–8*, demonstrating the presence of gene *4*

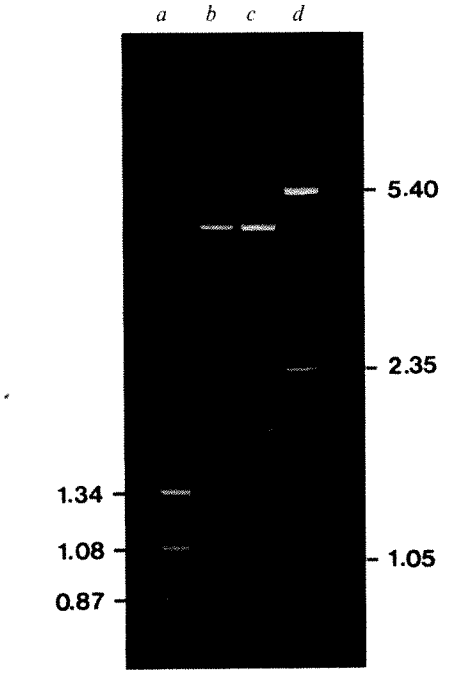


Fig. 1 Characterisation of the FPV gene *4* insert in pBR322 DNA. To prepare double-stranded cDNA from FPV RNA suitable for cloning, the total virion RNA from FPV (Rostock strain) was polyadenylated, and both cDNA strands were synthesised with reverse transcriptase, then treated with S_1 nuclease[18]. Approximately 0.2 μg cDNA was repaired in a 50-μl reaction containing: 50 mM Tris HCl *p*H 7.6, 10mM $MgCl_2$, 50 μM of each deoxynucleoside triphosphate, 1mM β-mercaptoethanol and 1 unit DNA polymerase I (Boehringer), for 10 min at 10°C, then ethanol precipitated. A synthetic *Hin*dIII linker (d[CCAAGCTTGG], Collaborative Research) was kinase-labelled and ligated to the gene mixture in a volume of 10 μl containing: 50 mM Tris HCl *p*H 7.6, 10mM $MgCl_2$, 20 mM dithiothreitol (DTT), 1 mM ATP, approximately 0.2 μg dsDNA, 2 μM 5′-^{32}P-labelled linker and 1 μl T_4 DNA ligase (New England Biolabs) for 7 h at 15 °C. After 7 and 23 h further linker was added to 2 μM, and at 23 h a further 1 μl ligase was added. Excess and unligated linkers were removed by *Hin*dIII digestion (30 units) at 37°C for 90 min, and chromatography through a G-150 column in 50 mM NaCl 0.2% SDS. The excluded peak containing genes was ethanol precipitated and used for ligation to pBR322 DNA as follows: 10 μg pBR322 DNA was digested with *Hin*dIII (25 units) and treated with bacterial alkaline phosphatase. After removing the enzymes with phenol, the linear dephosphorylated plasmid DNA (0.3 μg) was ligated to approximately 80 ng of gene mixture as above, but with 0.5 μl T_4 ligase in 20 μl for 16 h. 10 μl was withdrawn, and used to transform[21] *E. coli* K12 HB101. The culture was plated on L-agar in the presence of 100 μg ml^{-1} ampicillin. Plasmid DNA was purified from tetracycline-sensitive colonies and examined on a 1.4% agarose gel following *Hin*dIII restriction, an example of which is shown. *a*, *Hae*III cut ØX174 DNA markers in kilobase pairs; *b*, pBR322 + *Hin*dIII; *c*, pBR 322–FPV$_{4-10}$ + *Hin*dIII; *d*, *Hin*dIII cut PM2 DNA markers in kilobase pairs. To determine which FPV gene was inserted in each plasmid, ^{32}P-labelled gene probes were prepared and hybridised to plasmid DNA as follows: Plasmid DNA was first prepared by digesting 5 μg plasmid with *Eco*RI (12.5 units) and binding denatured 0.25 μg aliquots to small Millipore disks[23]. 20 μg FPV RNA was electrophoresed in a 3% polyacrylamide gel, *p*H 3.5 in 7M urea, and genes *1–3*, *4*, *5*, *6*, *7* and *8* eluted, incubated for 1 h at 0 °C in 0.1 M NaOH, and labelled with ^{32}P at the newly exposed 5′-ends. Each ^{32}P-labelled probe (approximately 3,000 c.p.m.) was incubated with the DNA-coated Millipore disk at 37°C for 16 h in 100 μl 50% formamide in 3×SSC, 0.1% SDS and 2 μg tRNA. After extensive washing (50% formamide plus 5×SSC, 2×SSC, 2×SSC plus 10 μg ml^{-1} pancreatic RNase and 2×SSC), the disks were dried and counted. Conditions for all restriction enzyme digestions were the same or very similar to those given in the catalogue of New England Biolabs Inc.

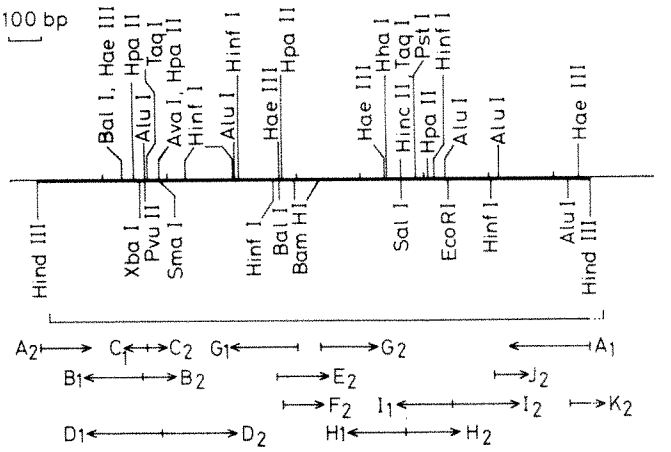


Fig. 2 Restriction enzyme map of the FPV gene *4* insert in pBR322. The insert is the thicker line, beneath which are shown the restriction sites labelled in the sequence analysis. The cloned gene *4* is 46 nucleotides shorter than full length gene *4* at the right hand end which corresponds to the 5′-terminal region of the virionRNA. The relative positions of the 'six base-pair' enzyme sites were first determined by standard mapping procedures. To locate sites for enzymes recognising 4 and 5 base pairs, the gene *4* insert (10 μg) was cut from the plasmid with *Hin*dIII (20 units) for 90 min at 37°C. After removal of enzyme, dephosphorylation and labelling of 5′-ends (Fig. 3), the insert was separated from the plasmid DNA by electrophoresis in a 1.4% agarose gel, and eluted[24]. Subsequent digestion with, for example, *Hin*fI gave at least six fragments as shown by polyacrylamide gel electrophoresis (5% acrylamide, 0.17% bisacrylamide in 50mM Tris-borate *p*H 8.3, 1 mM EDTA). The two *Hin*fI fragments containing the *Hin*dIII ends were identified from their ^{32}P label. The larger of these two was sensitive to *Sma*I, placing it at the left end of the insert. The positions of some of the other *Hin*fI sites were determined from the sensitivity of internal *Hin*fI fragments to *Sal*I and *Eco*RI. The distances sequenced are shown by arrows; those pointing to the left denote sequences derived from the virion RNA strand, and those pointing to the right from the cRNA strand. The vertical line interrupting each arrow indicates the restriction site used for labelling (see Fig. 3 legend for details). The dotted line in distance K_2 refers to the 5′-terminal 46 nucleotides of gene *4* missing from the plasmid. The line above the arrows indicates the position of full length gene *4* relative to the insert.

sequences in pBR322–FPV$_{4-10}$. This plasmid was chosen for sequence analysis as the insert was calculated[18] to be an almost full length copy of gene *4*.

Restriction enzyme mapping and sequence determination

As a first step to sequence analysis of pBR322–FPV$_{4-10}$, the cleavage sites for a number of restriction enzymes were mapped. Remarkably, all the six base-pair enzymes tested (except *Sac*I, *Hin*dIII and *Hpa*I) were found to cut the gene *4* insert at least once (Fig. 2). Some of these enzymes were used in the DNA sequence analysis (see Fig. 3 legend for details).

The lettered arrows in Fig. 2 refer to the distances sequenced and this procedure was usually done twice[22]. In all cases sequences from neighbouring restriction sites were overlapped by at least 15 nucleotides. It was useful to have the sequence on both strands at the overlap, where the bands on the sequencing gels (Fig. 3) were often close together. The few uncertainties were due either to compression[25] of bands at 'hairpin' loops or failure of a chemical reagent to cleave a phosphodiester bond[26] (at methylated C or A). These problems were overcome in all cases by sequencing the complementary DNA strand either in the opposite direction from a neighbouring restriction site, or in the same direction with 3′-end-labelled DNA[27] (see Fig. 4 legend for details).

We have confidence in the number of nucleotides present in the coding region because two protein synthesis reading frames are frequently blocked by nonsense triplets (Fig. 5), leaving one frame open to code for the protein sequence. Striking amino acid sequence homologies between FPV and human HA sequences[13,14] also provide some indication of accuracy in parts of the DNA sequence (Fig. 5).

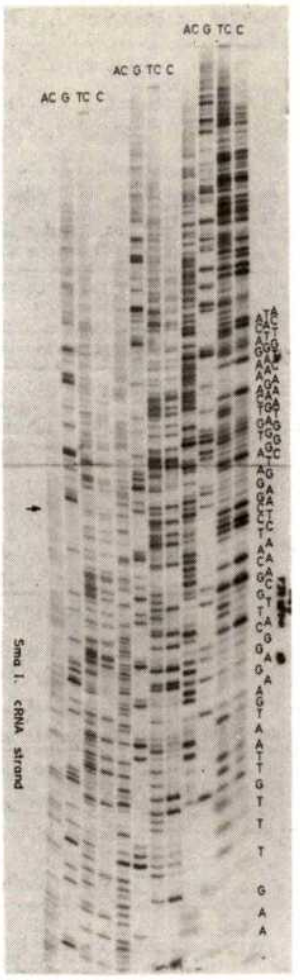


Fig. 3 Sequencing gel of mRNA strand at *Sma*I site. 20 μg pBR322–FPV_{4-10} was digested with *Sma*I (35 units) in 200 μl for 3 h at 37°C, then phenol/chloroform extracted and ethanol precipitated. The 5′-phosphates were removed with 10 μg bacterial alkaline phosphatase in 20 mM Tris *p*H 7.5, 0.1% SDS for 1 h at 37°C. After three phenol/chloroform and two ether extractions, the DNA was again precipitated in ethanol. Labelling was carried out using 200 μCi [γ-^{32}P]ATP (3,000 Ci mMole^{-1}) in a volume of 25 μl containing 3–4 units of T_4-polynucleotide kinase, 5 mM DTT, 0.1 mM spermidine, 50 mM Tris *p*H 9.5 and 10 mM $MgCl_2$. The enzyme was removed as described above for *Sma*I. The labelled DNA was then recut with *Hin*dIII and the two labelled fragments (0.367 and 1.36 kilobases) separated by electrophoresis in a 1.4% agarose gel. Following elution, the two fragments were subjected to the partial chemical degradations[22] and the products resolved in a 30×80×0.04 cm polyacrylamide gel[25] as shown above for the 1.36-kilobase fragment (distance D_2 in Fig. 2). Aliquots of the same sample were loaded at different times so as to read over 200 nucleotides (up to arrow). The other fragments used for sequence analysis were generated as follows: A_1, A_2, *Hin*dIII, label, recut *Eco*RI, isolate 0.447- and 1.28-kilobase fragments. B_1, B_2, *Xba*I, label, recut *Bam*HI, isolate 0.47- and 4.32-kilobase fragments. C_1, C_2, *Pvu*II, label, recut *Hin*dIII, isolate 0.321- and 1.41-kilobase fragments. E_2, J_2, *Hin*fI, label, recut *Sma*I plus *Hin*dIII plus *Pst*I, isolate *Hin*fI→*Pst*I and *Hin*fI→*Hin*dIII fragments (0.452 and 0.320 kilobases). F_2, *Bal*I, label, isolate 1.0-kilobase fragment, recut with *Sal*I, isolate 0.378-kilobase fragment, G_1, G_2, *Bam*HI (also labelled at 3′-ends with α-^{32}P-CTP using deoxynucleotidyl terminal transferase (Fig. 4)), label, recut *Pst*I, isolate 0.306- and 1.566-kilobase fragments. H_1, H_2, *Sal*I, label, recut *Sma*I, isolate 0.758- and 1.232-kilobase fragments, recut 1.232-kilobase fragment with *Bam*HI and isolate 0.96-kilobase fragment. I_1, I_2, *Eco*RI, label, recut *Ava*I, isolate 1.841- and 0.916-kilobase fragments. K_2, *Hae*III, label, isolate 0.608-kilobase fragment (*Hae*III (1,050)→*Hae*III (1,658) in Fig. 5), recut with *Alu*I, isolate 0.042-kilobase fragment (5% polyacrylamide gel as in Fig. 2 legend) [*Alu*I (1,616)→*Hae*III (1,658) in Fig. 5], label and use as a primer with FPV RNA as template (see Fig. 4 legend for details).

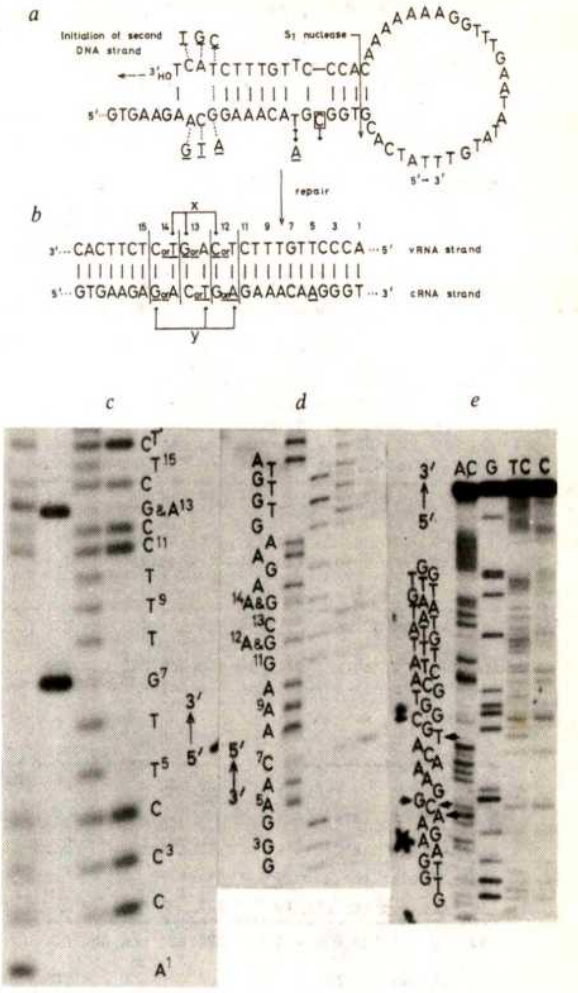
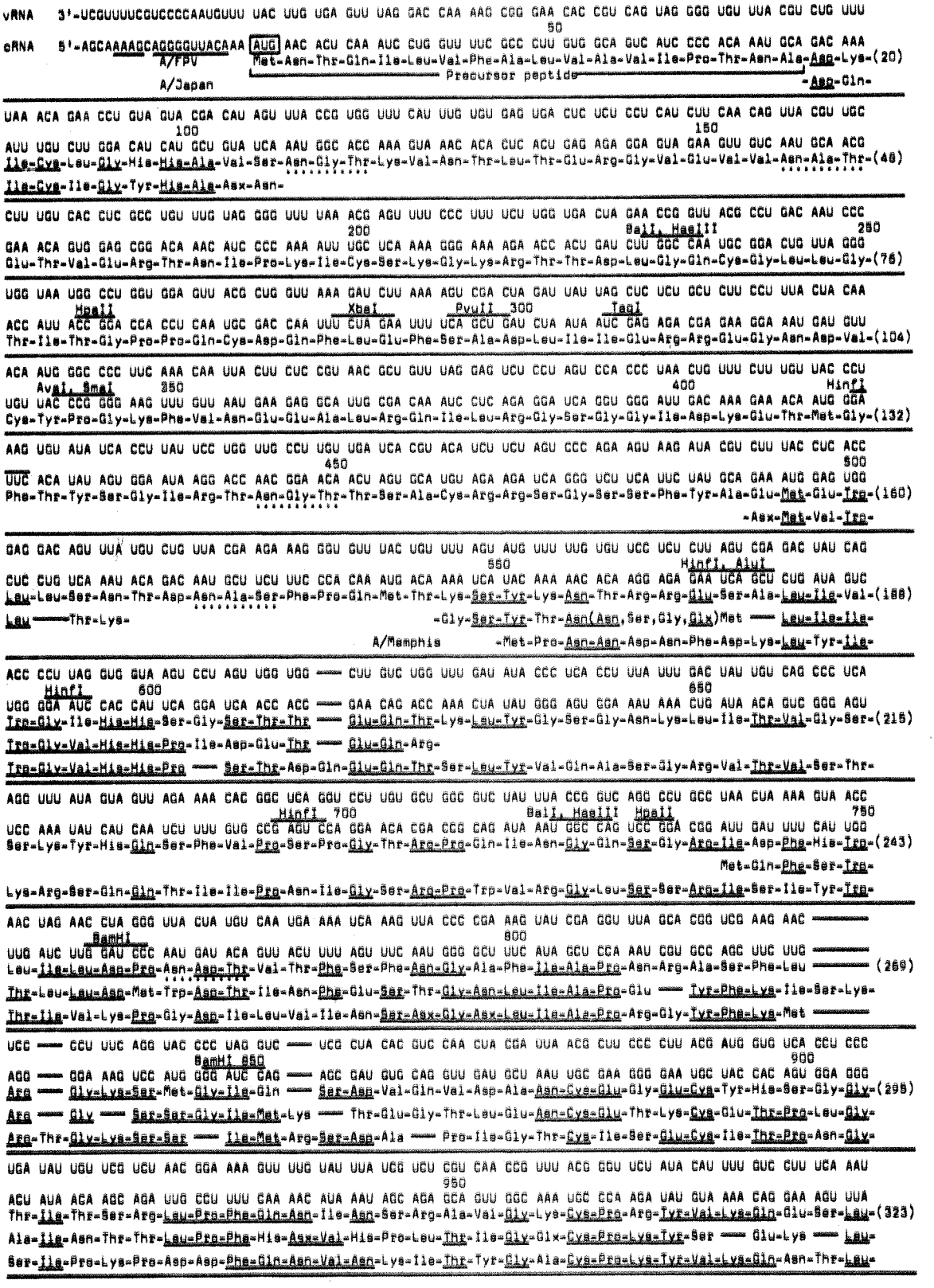


Fig. 4 Origin of the sequence at the right hand end of the gene *4* insert. *a*, The putative hairpin at the 5′-end of the first cDNA strand. *b*, The sequence found at the right hand end of the insert. The dotted lines in *a* refer to nucleotides that arose in the daughter molecules after the first round of plasmid replication. The result in *b* is two plasmids differing in positions 12–14, one with sequence x and the other sequence y. The complementary sequences for the right end of the insert going leftwards are shown in *c* (5′→3′) and *d* (3′→5′). Evidence for heterogeneity is indicated only for the purines. Sequencing in the 3′→5′ direction (*d*) was done by 3′-end labelling of DNA[27]. 20 μg of *Hin*dIII cut plasmid was heated at 90 °C for 5 min and rapidly cooled. The final incubation was: 0.4 mg ml^{-1} DNA, 5 mM Tris, 0.02 mM EDTA, 0.1 M sodium cacodylate *p*H 6.9, 1 mM $CoCl_2$, 0.1 mM DTT, 100 μCi[α-^{32}P]CTP(>350 Ci mMole^{-1}) and 5 units deoxynucleotidyl terminal transferase for 1 h at 37 °C, then for 30 min at 37 °C with an additional 5 units of enzyme. Excess rC was removed with 5 μg pancreatic RNase plus 20 μg tRNA. The enzymes were removed, the DNA was recut with *Eco*RI (50 units) and the 0.447-kilobase fragment was purified by electrophoresis in a 1.4% agarose gel. *e*, The sequence determined from the RNA of gene *4* by priming with a 42-nucleotide restriction fragment derived from the gene *4* insert (see Fig. 3 legend). This sequence spans the right end of the gene *4* insert (*b*), and the five differences with the cloned DNA sequences are arrowed. The 42 base-pair *Alu*I (1,616)→*Hae*III (1,658) fragment was generated from the 5′-^{32}P-labelled *Hae*III (1,050)→*Hae*III (1,658) fragment by *Alu*I digestion (50 units) and purified by 5% polyacrylamide gel electrophoresis. Numbers refer to the distance in nucleotides of the restriction site from the 3′-end of the virionRNA strand (Fig. 5).] The fragment was then labelled at the 5′-ends (Fig. 3) and ethanol precipitated with 15–20 μg FPV RNA. Denaturation was done at 80 °C for 2 min in 80% formamide, 0.01% SDS, 0.01M PIPES *p*H 6.5, 0.4 M NaCl, 1 mM EDTA and the mixture was annealed to the FPV RNA at 50 °C for 20 min. Following ethanol precipitation, cDNA synthesis was carried out with reverse transcriptase[16] and all four unlabelled deoxynucleoside triphosphates. To remove template RNA the cDNA/RNA was ethanol precipitated, briefly exposed to 0.3 M NaOH at 70 °C and neutralised at 20 °C. The large cDNA transcript was purified by 5% gel electrophoresis.



Evidence for repair and heterogeneity in cloned DNA

The first sequences to be established for the gene *4* insert were at the extremities (Fig. 2). The left end of the insert (adjacent to the *Eco*RI site of pBR322 DNA) was found to have the sequence corresponding exactly to the 3'-end of the virionRNA (5'-end of cRNA)[28], but the right hand end could not be matched with the published sequence at the 5'-end of the virionRNA[28]. Therefore an experiment was devised to determine how much of gene *4* was missing. A 42 base-pair restriction fragment from between the *Alu*I and *Hae*III sites nearest the right end of the insert (Fig. 2) was purified, labelled and used as a primer for reverse transcriptase in the presence of FPV RNA and all four unlabelled deoxynucleoside triphosphates[18] (see Fig. 4 legend for details). The strand corresponding to the cRNA presumably annealed to the RNA of gene *4* since the DNA product appeared as a single band in a 5% polyacrylamide gel (not shown). Its length was estimated to be about 140 nucleotides. The band was eluted and the DNA sequenced[22] (Fig. 4*e*). The

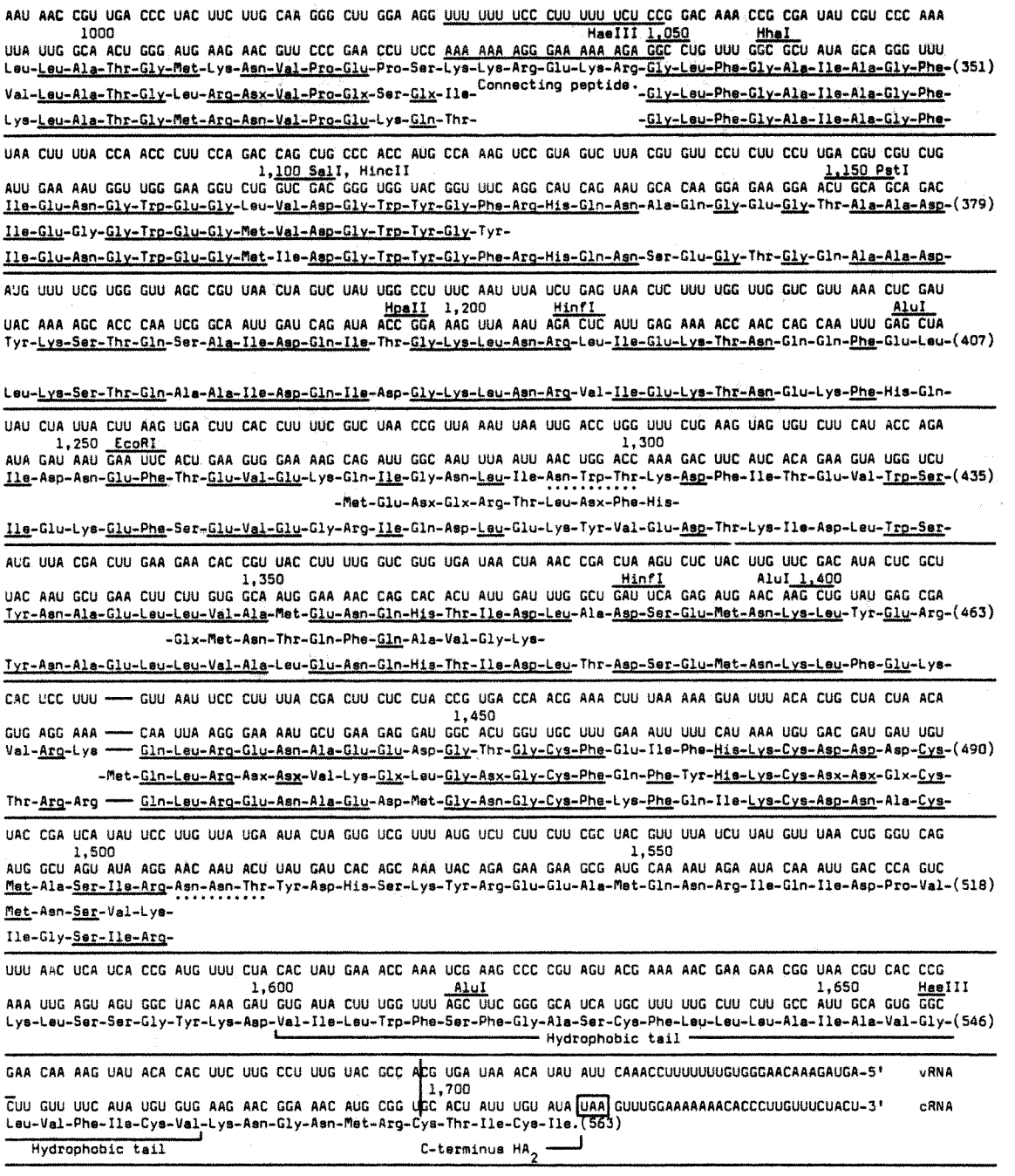


Fig. 5 Nucleotide sequence of the FPV HA gene and comparison of the predicted amino acid sequence of the HA with human HA sequences. The virus RNA (with the 3′-end to the left) is shown above the complementary RNA. Boxes show the initiation codon (AUG) and termination codon (UAA). The positions of the restriction enzyme sites (Fig. 2) found in the corresponding plasmid DNA are indicated by a bar above the cRNA. The heavy underlining near the initiation codon shows two areas of homology with prokaryotic ribosome binding sites. The underlined purine-rich sequence in the cRNA (nucleotides 1,029–1,049) spans the region coding for the peptide connecting HA_1 to HA_2. The vertical line to the left of the termination codon shows the position corresponding to the attachment of the *Hin*dIII linker to the incomplete DNA copy of gene *4*. To the left of this line is shown the actual sequence of the RNA gene (Fig. 4*e*). The predicted amino acid sequence of the immature FPV HA is immediately below the cRNA. The A/Japan/305/57 (H2N2) partial sequence is shown below the complete FPV sequence, and the third line has the A/Memphis/102/72 (H3N2) partial sequence. In maximising homology (underlining) the sequences of these HAs have been aligned according to the positions of the cysteines. Dotted lines below the FPV HA sequence indicate asparagines which may be glycosylated in the mature HA.

23 nucleotides at its 3′-terminal end are complementary to the published sequence of 23 nucleotides at the 5′-end of FPV gene *4*, demonstrating that the strong transcription stop had occurred at the 5′-end of the template RNA[28]. The results show that the FPV gene *4* insert is only 46 nucleotides shorter than the full length gene (Fig. 5). The sequence of 70 nucleotides at the 5′-terminus derived in this way is identical to the sequence determined for the 5′-end of gene *4* by rapid RNA sequencing[53]. However, five differences were found between the sequence deduced by these two independent methods (Fig. 4*e*) and the sequence near the right hand end of the insert determined on both cloned DNA strands (Fig. 4*c*, *d*). These differences (arrowed in Fig. 4*e*) are from top to bottom: absence of a G·C pair in the cloned DNA (Fig. 4*c*, *d*, between positions 3 and 4); an A instead of a T in the cloned DNA (Fig. 4*d*, position 5); and sequence heterogeneity in three positions in the cloned DNA. Both an A and a G are seen at position 13 (Fig. 4*c*) and at positions 12 and 14 (Fig. 4*d*), whereas elsewhere on these sequencing gels cuts are made only at A or G. Heterogeneity in the corresponding pyrimidines is not so obvious since cuts after

C are normally made in both pyrimidine-specific reactions[22].

It is extremely unlikely that these base changes resulted from reverse transcriptase errors, because the conditions of reverse transcription of FPV RNA using the 42-base-pair primer were identical to those used in synthesis of double-stranded DNA for cloning. We suggest instead the following model to explain why the base changes occurred. As with other cDNAs, a hairpin will form and prime the synthesis of the second cDNA strand[29]. The structure in Fig. 4*a* is the only one that can account for the loss of 46 nucleotides from the 5'-end of the gene, provided that the following assumptions are made. First, in the molecule which was cloned the three single-stranded 'bubbles', but not the large terminal loop, survived S_1 nuclease treatment, and second, reverse transcriptase stabilised the interaction of the 3'-terminal T with an A on the opposite strand so that synthesis of the second cDNA strand could commence.

In the proposed hairpin structure (Fig. 4*a*) there are three regions where mismatching occurs, and these regions coincide exactly with the five base changes found in the cloned DNA. The largest 'bubble' (positions 12–14) corresponds to the three positions where sequence heterogeneity is observed (Fig. 4*c*, *d*). The heterogeneity reflects the presence of two plasmids in the bacteria, and it seems probable that these would have arisen after the first round of DNA replication, since it is highly unlikely that one bacterial cell would have been transformed by two plasmid molecules of identical lengths derived from a heterogenous mixture of reverse transcripts[18]. Thus, the mismatched AC pairs at positions 13 and 14 (Fig. 4a) would segregate to a TA pair in one new DNA molecule and a CG pair in the other. Likewise, the GT pair at position 12 becomes a GC in one new DNA molecule and a TA in the other.

In the other two regions excisions could have taken place to restore a perfect duplex (Fig. 4*b*). Thus, the extra C residue nearest the S_1 cleavage site is excised, and two residues to the left (position 5) a T in the strand corresponding to the cRNA is replaced with an A, which can now base-pair with the T on the opposite DNA strand. In contrast to positions 12–14, there is no evidence of sequence heterogeneity, suggesting that the excisions occurred before replication.

The nucleotide sequence of FPV gene *4*

The FPV gene *4* is 1,742 nucleotides long (Fig. 5). This is about 300 nucleotides shorter than a previous estimate[18] of 2,060 nucleotides based on migration rate of RNA in formamide–polyacrylamide gels. The coding region begins with an AUG and is terminated by a single nonsense codon (UAA). Around nucleotides 1,030–1,050 there is an unusual sequence in the mRNA—an uninterrupted stretch of purines most of which code for the peptide connecting the two HA subunits, HA_1 and HA_2 (ref. 13).

During *in vivo* transcription of viral RNA, host mRNAs prime the synthesis of influenza mRNAs adding an extra 10–15 nucleotides to each 5'-end[30] (not shown). At the other end, transcription terminates prematurely[31], probably at the U_7 sequence 17–23 nucleotides from the 5'-end of the virionRNA template. Thus the 5'- and 3'-non-coding regions (including the UAA) are 31–36 and approximately 9 nucleotides long, respectively. The length of the 3' region is therefore short compared with the corresponding region of other mRNAs found in eukaryotic cells[26,29,32]. A putative signal sequence 5'AAUAAA3' has been found in the 3'-non-coding region of many different types of eukaryotic mRNA[26,29,32]. It is absent from FPV gene *4* (Fig. 5) and this resembles the situation in some picornaviruses[33] and one of the mRNAs of vesicular stomatitis virus[34].

The 3'-ends of the cRNA and virionRNA of gene *4* can be aligned so that 12 out of 15 nucleotides are the same. This is significant as a possible signal for the initiation of replication on both these RNAs as has already been noted[28,31]. Homology between 3'-ends also means that the 5'- and 3'-ends of either virionRNA or cRNA are complementary[28], but there is no known function for such an interaction.

A striking feature of the 5'-non-coding region near the AUG (underlined in Fig. 5) is the homology with some prokaryotic ribosome binding sites. These include the R17 replicase[35], T_7 early mRNA[36] and *E. coli* Trp E sites[37]. In FPV gene *4*, there is a characteristic purine-rich sequence, AGGGG, which interacts with the 3'-end of 16S rRNA in prokaryotes[38], followed immediately by UUACA. The latter sequence is present in the prokaryotic sites as UUAC and U-ACA. There is even greater homology between the gene *4* site and a spurious ribosome binding site in Qβ RNA which lacks an AUG[39]. Here at least 12 out of 16 nucleotides are the same. Support for the idea that the gene *4* site is prokaryotic in character comes from experiments on the expression of FPV gene *4* in *E. coli*[40].

Codon usage

The choice between degenerate codons in many mRNAs is frequently non-random, with markedly different preferences in different mRNAs[26,29,32]. In some mRNAs, the most frequently used codons correlate with the abundant species of isoaccepting tRNA[41], while in others the usage reflects the base composition[42]. FPV gene *4* is U-rich (34.3%) and has a low G content (18.5%). Reflecting this, there is a significant bias for codons with A in the third position (notably GAA, AGA and AAA), and against codons with third position C (Table 1).

Gene *4* has an overall deficiency of CG relative to the other 15 dinucleotides. This is not due only to the low G content, since GG sequences are almost twice as abundant. Again reflecting the base composition of the virionRNA, codons with CG in first and second or second and third position rarely occur (Table 1). There is also a deficiency of CG in the eukaryotic genome[43]. In this case it has been suggested that the frequency of CG has been kept to a minimum since cytosine in CG is a major site of DNA methylation and methylated nucleotides are 'hot spots' for mutation[32]. But this cannot be true for influenza, an RNA virus with no known DNA intermediate in its replication.

Amino acid sequence organisation of the FPV haemagglutinin

The immature FPV HA consists of 563 amino acids, of which 18 form the N-terminal 'pre-peptide', 319 are in HA_1, 5 are in a connecting peptide and 221 are in HA_2 (Fig. 5). The amino acid composition shows similarities to human haemagglutinins; for example in the high proline content of HA_1 compared to HA_2, and in the number and distribution of cysteine residues[13,14]. The positions of the cysteines have been highly conserved, perhaps to help give each molecule a basically similar overall shape by disulphide bridging.

Existing evidence suggests that immature HA has a short N-terminal leader sequence which is cleaved off when the process of membrane insertion is complete[14]. The DNA sequence predicts that the FPV leader consists of 18 amino acids, none of which is polar (Fig. 5). From the fifth residue, there is a core of 11 consecutive hydrophobic amino acids. The leader sequence of another influenza A haemagglutinin (RI/5$^-$/57) is 15 residues in length and shows almost no sequence homology with the FPV leader, but like FPV has an uninterrupted sequence of hydrophobic amino acids[44]. The size and non-polar character of these N-terminal peptides is very similar in other secreted proteins[45], which lends strong support to the signal peptide hypothesis[46]. This states that the process of membrane transport is initiated by insertion of a hydrophobic pre-peptide, which is subsequently cleaved off.

Cleavage of HA into HA_1 and HA_2 usually takes place either on smooth internal membranes or at the plasma membrane[47]. This step is essential for infectivity[9] and apparently also for pathogenicity[48]. It is carried out by an unknown host protease with a similar specificity to trypsin[47]. In FPV, the sequence around the cleavage site is Lys-Lys-Arg-Glu-Lys-Arg-Gly (Fig. 5), where the first residue becomes the C-terminus of HA_1 and the last residue the N-terminus of HA_2. The basic nature of these residues accounts for the *in vitro* trypsin cleavage of HA

into HA_1 and HA_2, but it is not known whether only some or all the basic residues are cut either *in vitro* or *in vivo*. In contrast to FPV, the C-terminal amino acid of HA_1 of A/Japan/305/57 is isoleucine[14] and A/Memphis/102/72 has C-terminal threonine[13]. These differences could explain why the susceptibility of HA to cleavage depends on the virus strain[47] as well as on the cell type[48].

In human influenza A strains, the C-terminal part of HA_2 has not yet been sequenced owing to the insolubility of the peptides derived from it, indicating the presence of an extensive hydrophobic region[13,14]. In FPV this begins at residue 527 and extends for 26 amino acids to residue 552 (Fig. 5). Of these, 20 residues are hydrophobic, and are probably involved in anchoring the HA to the viral lipoprotein envelope[14].

There are five potential sites for carbohydrate attachment in HA_1 and two in HA_2 (Fig. 5). This conclusion is based on the presence of the sequences Asn-X-Ser and Asn-X-Thr, which are known to occur in other glycoproteins (including haemagglutinins[13,14]) at sites of N-glycosidic linkage of asparagine to oligosaccharides[14]. Previous studies on the HA from FPV (Dutch) have indicated that there are five or six separate oligosaccharide units, but their exact location was not determined[49]. In A/Japan/305/57, the approximate location of carbohydrate side chains is known to be residues 11, 23, 192 and 303 in mature HA_1 and very near the bromelain cleavage site in HA_2 (ref. 14). In FPV, the sites at residues 30 and 46 in HA_1, and residue 496 in HA_2 seem to correspond to the known A/Japan sites at 11 and 23 in mature HA_1 and the single site in HA_2, respectively.

In Fig. 5, the FPV HA sequence is compared with published partial HA sequences for an H2N2 strain (A/Japan/305/57) and an H3N2 strain[13,14] (A/Memphis/102/72). There is considerable sequence homology (38%) between all strains. This occurs to an even greater extent between FPV and Japan (48%), Memphis and Japan (50%) and FPV and Memphis (55.5%). Conservation of sequence appears to be greater in HA_2 than HA_1, which is not surprising as only HA_1 is thought to undergo antigenic variation.

As pointed out previously, the N-terminal 24 residues of several HA_2 molecules are highly conserved[14], and this now extends to FPV. The cleavage into HA_1 and HA_2 may expose the hydrophobic sequence at the N-terminus of HA_2, which then enables the HA to penetrate the cell membrane[14]. In at least 63% of positions where there is an amino acid difference between any two HAs (Fig. 5), the change can be accounted for by a single nucleotide substitution. This is significantly higher than the value of 38% for all possible pairs of amino acids. This consideration, together with the overlapping nature of the homologies between the three HAs (Fig. 5), implies that the corresponding HA genes have evolved from one and not from two or more distinct ancestors.

Concluding remarks

Gene *4* of FPV is the first influenza virus gene to be completely sequenced and it predicts for the first time the complete amino acid sequence of a viral HA. The gene has some interesting features. The most notable of these are seen in the mRNA—a short 3′ non-coding region, a prokaryotic-like ribosome binding site and an apparent lack of introns.

The sequence analysis of one end of the cloned gene *4* shows clearly that base changes and a base deletion occurred during the cloning process. The model to account for these changes is compelling, implying that because the changes are related to the hairpin at the 5′-end of the first cDNA strand they will not be found elsewhere in the synthetic gene. The results in any event indicate the importance of sequencing RNA independently of cloned DNA.

The HA of influenza viruses is a remarkable multifunctional protein. Our results and those of others have built up a picture of several functionally important domains in the molecule. These are the three hydrophobic regions with distinct functions, the connecting peptide and the carbohydrate attachment sites.

The region of HA that interacts with cell receptors at an early stage in infection remains to be determined, as do the antigenic sites, now known to be in the N-terminal half of HA_1 in a Hong Kong strain[50]. The FPV sequence does, however, provide a framework for determining these sites in combination with other approaches. For example, it will now be possible to determine the primary structure of HA genes from influenza strains that differ only in their reactivity towards monoclonal antibodies[51]. A single nucleotide change would show which amino acid had changed and pinpoint the antigenic site. Ultimately, these methods may enable us to predict which amino acid changes will produce influenza viruses capable of causing epidemics.

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Transposable mating type genes in *Saccharomyces cerevisiae*

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A functional copy of the α mating type gene of Saccharomyces cerevisiae *has been cloned by transformation in yeast. Using the Southern blotting procedure it has been shown that three distinct genetic loci implicated in mating type interconversion* (HML, HMR *and* MAT) *contain sequences homologous to the cloned fragment. The restriction fragment associated with each locus exhibits a characteristic size which can be correlated with the mating type allele present at that locus. The characteristic size difference between the* **a** *and α genetic elements made it possible to demonstrate that the homothallic interconversion of mating types in this yeast occurs by DNA rearrangement as proposed in the 'cassette hypothesis'.*

IN the life cycle of homothallic baker's yeast a simple example of cell differentiation occurs in which haploid cells of one mating type (**a** or α) give rise to progeny of the opposite type during mitotic growth. Subsequent conjugation between siblings of opposite cell type establishes the **a**/α diploid phase. Genetic observations on this regulatory switching process have led to the proposal that the mating type genes reside on translocatable DNA elements and that switches between cell types are accomplished by transposition of these elements[1,2]. Although a great deal of genetic evidence has been obtained in support of this 'cassette model' for mating type interconversion, direct demonstration of the proposed transposition mechanism requires identification and cloning of the DNA sequences involved. Using recently developed methods for yeast transformation, we have isolated a copy of the α mating type gene and have used this clone as a hybridisation probe to analyse the arrangement of the mating type genes in the yeast genome and to document that genetic rearrangements are involved in the mating type interconversion process. Our analysis is consistent with the prediction of the cassette model that silent copies of the mating type genes reside at the *HML* and *HMR* loci on chromosome III and that replicas of these unexpressed sequences are translocated to the mating type locus when switching occurs.

Background of the cassette hypothesis

The mating behaviour of *Saccharomyces* yeasts is controlled by the mating type locus (*MAT*) which has two alleles (*MAT***a** and *MAT*α). Cells expressing *MAT***a** (mating type **a**) conjugate readily with cells expressing *MAT*α (mating type α) to create *MAT***a**/*MAT*α diploids. In heterothallic (*ho*) strains the mating types are stable and interconvert only rarely (10^{-6}) while in homothallic (*HO*) strains interconversion of mating types occurs as often as every cell division[3]. A haploid *HO* spore of one mating type can divide twice to yield four daughter cells, two exhibiting the parental mating type and two of the opposite cell type. Progeny of opposite mating type then conjugate to establish *MAT***a**/*MAT*α diploid cell lines in which both switching and mating functions are shut off. This switching process is due to heritable but reversible alterations of the *MAT* locus, and the continued presence of the *HO* gene is not required for the maintenance of the altered state of that locus[4–7].

Two other genetic loci, *HM***a** and *HM*α are also required for switching[8–10]. The genetic nomenclature originally associated with these loci has become somewhat confusing in the light of the function provided by these genes, as described below. We will use here a simpler system which was proposed at the International Congress on Yeast Genetics in 1978. Both forms are presented in Table 1. The *HML* locus (formerly *HM***a**) on the left arm of chromosome III as it is conventionally drawn (see Fig. 1), has two naturally-occurring co-dominant alleles: *HML***a** and *HML*α. Similarly, the *HMR* locus on the right arm of the chromosome has two codominant alleles *HMR***a** and *HMR*α. Either *HML***a** or *HMR***a** is required for the activation of the **a** mating type gene at the mating type locus[11]. Similarly, either *HML*α or *HMR*α is required for the activation of the α mating type gene at the mating type locus[12].

As an explanation of the heritable changes observed at the *MAT* locus during switching, Hicks, Strathern and Herskowitz[1] proposed the 'cassette model' for mating type interconversion in which changes of cell type occur through transposition of **a** and α mating type genes to the *MAT* locus from unexpressed storage sites at *HML* and *HMR*. This model provides a molecular explanation of the heritability of the switched *MAT* alleles and accounts for the striking observation that cells carrying a mutant *mat*α^- (sterile) allele can switch to *MAT***a**$^+$ and then 'healed' to *MAT*α^+ (fertile) within three generations in the presence of the *HO* gene. This healing occurs with a variety of *mat*α^- and *mat***a**$^-$ mutations[13–15]. Therefore, cells capable of switching must contain reserve copies of *MAT***a** and *MAT*α information in the genome. In the cassette model, the *HML* and *HMR* loci are proposed to contain normally unexpressed copies of the mating type gene; the *HML*α and *HMR*α alleles corresponding to α information and the *HML***a** and *HMR***a** alleles to **a** information. A change of cell type is thus viewed as a loss of previously expressed DNA from *MAT* concomitant with an insertion of a replica (cassette) of **a** or α information derived from the *HML* or *HMR* loci. This scheme is shown in Fig. 1. Several predictions of the cassette model have been recently verified: (1) a number of mutations have been characterised which apparently lead to the expression of **a** or α information at the normally silent *HML* and *HMR* loci[15–18]; (2) chromosome rearrangements have been identified which lead to the activation of *HML*α or *HMR***a** by fusion to *MAT*[19]; and (3) mutations in the *HML* and *HMR* loci have been transposed to *MAT* by switching[20,21].

Table 1 Nomenclature of genes involved in switching *MAT**

Old	New
HMa	*HMLα*
hma	*HMLa*
HMα	*HMRa*
hmα	*HMRα*

Capital letters are used for both alleles of each locus to indicate codominance. Recessive mutant alleles are indicated by lower case designations (for example, *hmra*$^-$).

* Data compiled from Harashima *et al.*[8] and Naumov and Tolstorukov[9].

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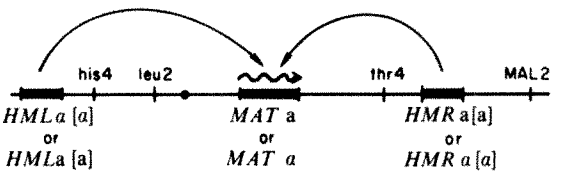


Fig. 1 Map of chromosome III and the cassette model for mating type interconversion. *HML* and *HMR* are sites which contain unexpressed copies of the **a** and α genes. Most laboratory strains exhibit the *HMLα* and *HMR***a** arrangement of storage genes. During interconversion a copy of one of the silent genes is transposed to the *MAT* locus region where it is expressed. The fate of the original *MAT* allele is not clear but it has not been shown to reappear in the genome and is presumably lost.

Identification of a cloned α gene by transformation in yeast

The cassette model makes strong predictions concerning the physical arrangement of the **a** and α DNA sequences in the genome. Clearly, if this model is correct, an α gene at *MAT* should have an homologous counterpart at *HMLα* and *HMRα*. Furthermore, as **a** and α are distinct codominant genes, there must be some physical difference between the *MAT***a** and *MATα* alleles. The cassette model predicts that a similar difference should distinguish the *HML***a** and *HMLα* alleles and the *HMR***a** and *HMRα* alleles. To test these predictions, it was necessary to identify and clone a mating type gene and use it as a hybridisation probe to examine the arrangement of homologous **a** and α sequences in the genome. As there is no easily obtainable physical probe for a yeast mating type gene, we used recently developed techniques for yeast transformation to isolate a mating type α gene by its ability to complement a defective mating type locus in yeast.

The vehicle that we have constructed for cloning yeast genes consists of a 1.4×10^6 molecular weight *Eco*RI fragment of the B form of *Scp*I cloned into the *Eco*RI site of the bacterial vector pBR322 along with a *Pst*I fragment containing the yeast *LEU*2 gene inserted into a *Pst*I site in *Scp*I sequence. This plasmid, designated YEp13 in accord with the nomenclature of Davis *et al.*[22], confers resistance to ampicillin and tetracycline in *Escherichia coli*, complements the bacterial *leu*B6 and yeast *leu*2 mutations, replicates as a plasmid in both organisms and transforms yeast at high frequency[23]. A library of cloned yeast DNA molecules was constructed by ligating *Bam*HI restriction fragments from α haploid strain DC56 into the unique *Bam*HI site in the Tet^R determinant of YEp13 and transforming *E. coli* C600 and screening for Amp^R Leu^+ Tet^S colonies. These colonies containing cloned yeast DNA were divided into subsets of 200 yeast clones each and the clones were amplified by standard procedures as described previously[24].

To detect a plasmid containing the yeast α mating type gene, pooled plasmid DNA was used to transform a yeast strain (DC49) containing a defective *mat***a**$^-$ allele along with a non-reverting double mutation in the *leu*2 gene[25]. The recessive *mat***a**$^-$ allele was used so that transformants containing an α plasmid would exhibit the α mating phenotype (*MATα*/*mat***a**$^- = \alpha$). Transformation was performed as described previously[25] and Leu^+ colonies were screened for α mating phenotype.

One subset of the clone library yielded Leu^+ transformants of DC 56 with the α mating phenotype. Several such isolates were purified as single colonies and DNA was prepared from each by the method of Cryer *et al.*[26]. The isolated DNA was used to re-transform the original bacterial strain (C600) to Amp^R Leu^+, and plasmid DNA was prepared from the bacterial transformants. When the plasmid isolated from this bacterial strain was used to re-transform yeast strain DC49, 100% of the Leu^+ transformants exhibited the α phenotype. This plasmid, designated YEp13-7, was shown by restriction mapping to consist of the original vector plus an insert of four *Bam*HI restriction fragments totalling about 16 kilobase pairs. We demonstrated that only one of those *Bam*HI fragments is required for the α phenotype by subcloning it on YEp13 and transforming DC49. This 6.6-kilobase pair yeast fragment is designated *Bam*26 and is outlined in Fig. 2. The α Leu^+ transformants of DC49 with YEp13-26 (YEp13 plus *Bam*26) were subsequently crossed with a *leu*2$^-$ haploid tester strains. The resultant diploids were able to undergo meiosis and sporulation, indicating that the YEp13-26 plasmid supplies not only the functions necessary for α mating ability but the α sporulation functions as well. A restriction map of the insert in YEp13-26 is presented in Fig. 2. Leu^- mitotic segregants from these strains (the result of loss of the plasmid) concomitantly lost both α mating and sporulation functions.

Physical mapping of the MAT locus

The results described above were consistent with the notion that plasmid YEp13-26 contains a functional α gene but would have also been obtained if the cloned insert somehow disturbed the normal regulatory mechanisms in the transformed cell, allowing an unexpressed copy of α (that is, the copy proposed to be present at *HMLα*) in the DC49 genome to be expressed constitutively. Fortunately, it was possible to identify the cloned DNA as a nucleotide sequence homologous to the mating type locus by mapping the naturally-occurring differences in restriction patterns between **a** and α strains. This restriction fragment polymorphism was observed when the YEp13-26 plasmid was used as a hybridisation probe against Southern blots of restricted DNA from a series of different yeast strains. By crossing strains carrying different versions of a polymorphic restriction fragment and analysing DNA from individual meiotic segregants, the size difference (and hence the cloned DNA sequence used as a probe) can be mapped against known genetic markers. This strategy has previously been used to map a tRNA clone to the *SUP*4 locus of yeast[27] and we have used it to identify a clone containing the yeast *can*1 gene[23].

The α/**a** diploid strain XJ777 used for mapping the cloned α gene was made by crossing the haploid strain DC5 (**a**) and DC55 (α). The complete genotypes of the strains are presented in Table 2. Tetrads were dissected and genetic markers scored by standard techniques[28]. DNA was then isolated from the parent strains and from individual spore clones derived from seven tetrads, digested with restriction endonuclease and displayed on agarose gels for Southern blotting[29]. The blots were probed with ^{32}P-labelled DNA from plasmid pBR322-26.2 which contains the larger *Eco*RI-*Bam*HI subfragment of YEp13-26 (Fig. 2). Figure 3 is an autoradiograph of a blot containing two representative tetrads from XJ777 digested with restriction endonuclease *Hin*dIII. There are two striking features of the hybridisation pattern. First, the hybridisation probe, which contains a *Hin*dIII site and therefore might be expected to hydridise to two chromosomal *Hin*dIII fragments, actually hybridises strongly to four such fragments. Second, two of the

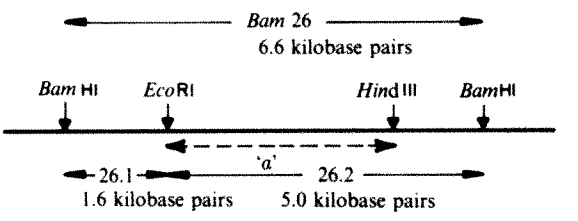


Fig. 2 Restriction map of the *Bam*HI fragment containing α functions. The dotted arrow represents the limits of the α sequence which are homologous with restriction fragments containing *MAT* and *HMR*.

Table 2 Genotypes of yeast strains used

DC49	*HMLα leu2-3, 2-112 mata HMRa his3 can1*
DC5	*HMLα leu2-3, 2-112 MATa HMRa his3 can1*
DC55	*HMLα MATα HMRa lys2 arg4 tyr1*
DC89	*HMLα his4 leu2 mata⁻ thr4 hmra⁻ met13 lys1 trp1* *HMRa + + mata⁻ + hmra⁻ met13 lys1 trp1*
DC90	*hmla⁻ his4 leu2 mata⁻ thr4 HMRα MAL2 lys1 met13 ade6* *hmla⁻ + + mata⁻ + HMLa ma12 + + +*
X10-1B	*HMLα MATα HMRa HO his5 ade5 met4 met13 ura4* *HMLα MATa HMRa HO his5 ade5 met4 met13 ura4*
JH204	*HMLα MATa HMRα HO his5 ade5 met4 met13 ura4* *HMLα MATα HMRα HO his5 ade5 met4 met13 ura4*
JH210	*HMLa MATa HMRa HO his5 ade5 met4 met13 ura4*

four *Hin*dIII fragments exhibit a length polymorphism which segregates in mendelian fashion. The polymorphic band C exhibits 100% co-segregation with mating type. All seven tetrads had the parental arrangement of mating type relative to the band C polymorphism (complete data not shown). This band shift, linked to the *MAT* locus, which is consistent with a size difference of ~200 base pairs, was also observed when tetrad DNA was cut with *Eco*RI or *Bam*HI. In each case the fragment corresponding to the *MATa* allele seemed to be ~200 base pairs smaller than the *MATα* fragment. Thus, we conclude that the insert in YEp13-26 is homologous to a DNA restriction fragment tightly linked to the *MAT* locus, providing strong additional evidence that this plasmid contains an α structural gene. The segregation of the band A polymorphism is not correlated with mating type and reflects a naturally occurring strain difference at a locus unlinked to *MAT* as described below.

The observation that the *MATα*-containing restriction fragment is ~200 base pairs larger than the fragment containing *MATa* when a variety of restriction enzymes are used indicates that the **a** and α strains used in this mapping differ by a small net insertion at or near the *MAT* locus. We demonstrated that this net size difference reflects a difference in the size of the *MATa* and *MATα* genes by taking a heterothallic yeast strain through a series of changes of cell type: *MATa* to *MATα* to *MATa* to *MATα*. DNA was made from each step and analysed as in Fig. 3. The switches from **a** to α resulted in 200-base pair increases in the *Hin*dIII fragment containing *MAT* and the α to **a** switch resulted in a net 200-base pair decrease (data not shown).

Mapping of *HMR* and *HML* fragments

Two of the additional hybridisation bands in Fig. 3 may be explained in terms of the cassette model; that is, they should represent restriction fragments corresponding to the *HML* and *HMR* loci. We therefore used the same mapping procedure to determine the identity of these bands. As *HML* and *HMR* are normally unexpressed, mapping these loci requires the construction of special strains in which their genotypes can be scored. We have used the *mar*1⁻ allele, first identified by Klar *et al.*[16], to construct mapping strains in which the *HML* and *HMR* alleles are expressed. The *MAR*1 gene product has been proposed to keep the information at the *HML* and *HMR* silent by negative control. In the *mar*1⁻ background, the *HML* and *HMR* loci act as if they contain active mating type genes. A normal haploid strain of genotype *HMLα MATα HMRa* becomes a non-mater in the presence of *mar*1⁻ because both **a** and α mating type genes are expressed. A strain of genotype *mar*1⁻ *HMLα mata⁻ hmra⁻*, on the other hand, behaves as an α-mater because *HMLα* is expressed and the other two loci are defective. In such a strain, the α mating type maps to the *HML* locus, distal to *his*4 (see Fig. 1) rather than to *MAT* (unpublished results). By constructing *mar*1⁻ strains with the appropriate arrangement of mutant and normal mating type alleles, as shown in Table 1, it is possible to place the only functional mating type genes at *HML* (*HMLa*/*HMLα*, strain DC89) or at *HMR* (*HMRa*/*HMRα*, strain DC90). The segregation of the **a** and α phenotypes in these strains thus reflects the segregation of the alleles at *HML* (DC89) or *HMR* (DC90) rather than *MAT*. Segregants from these strains can then be screened by Southern blot analysis as described above. The strategy used in the construction of these mapping strains will be described elsewhere (J.H., J.N.S. and A.J.S.K., in preparation).

In the cassette model *HML* and *HMR* are sources of DNA sequences that become substituted into *MAT*. If, as suggested, *HMRa* is an unexpressed **a** sequence and *HMRα* is unexpressed α, then a strain segregating those alleles (DC90) should exhibit a ~200-base pair polymorphism as demonstrated for *MAT*. Specifically, *HMRα* and *HMLα* should be ~200 base pairs larger than their *HMRa* or *HMLa* alleles. Figure 4 shows autoradiographs of two tetrads derived from diploids in which *HML* or *HMR* has been made heterozygous for active **a** and α regions. The genotypes of these diploids are presented in Table 2. Once again, alleles at these loci are associated with a restriction fragment polymorphism. In Fig. 4*a* the upper band exhibitis the polymorphism co-segregating with the *HMLa* and *HMLα* alleles. As in the case of the *MAT* locus, the **a** (*HMLa*) allele

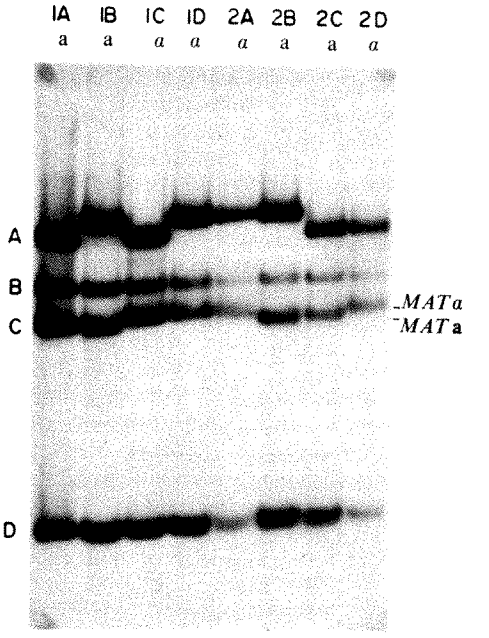


Fig. 3 Autoradiograph of two meiotic tetrads derived from crossing DC5 (**a**) and DC55 (α). DNA was cut with *Hin*dIII, blotted on nitrocellulose and hybridised with pBR322–26.2. The size shift in band C correlates with the segregation of mating type. Yeast culture media and standard yeast techniques have been described previously[3,7]. Yeast strains used in this work are presented in Table 1. Plasmids were propagated in *E. coli* B strain C600 (*leu*B *thr pro*) grown in Luria broth supplemented with ampicillin (100 μg ml^{-1}) or tetracycline (25 μg ml^{-1}) as required or on M9TP (1XM9 salts[30], 1% glucose, 1 mM $MgCl_2$, 20 μg ml^{-1} thiamine, 100 μg ml^{-1} threonine, and 10 μg ml^{-1} proline). *E. coli* strains were grown in M9CAA (1XM9 salts, 1% casaminoacids, 1% glucose, 1 mM $MgCl_2$, and 20 μg ml^{-1} thiamine) for large scale plasmid preparations. Yeast DNA was isolated from appropriate strains by the method of Cryer *et al.*[26] using equilibrium centrifugation in CsCl as a final purification step. Plasmid DNA was isolated from *E. coli* by standard procedures. Restriction endonuclease digestions, DNA ligation reactions and DNA labelling with ^{32}P were performed as described previously[23]. Fractionation of DNA was accomplished by electrophoresis on horizontal agarose slab gels poured and run in 40 mM Tris-acetate, *p*H 7.6, 1 mM EDTA, and 1.0 μg ml^{-1} ethidium bromide. Transfer of DNA from agarose gels to nitrocellulose filters was affected by the method of Southern[29]. Hybridisation of labelled DNA to DNA immobilised on nitrocellulose filters and transformation of yeast cells with purified plasmid DNA was performed as described elsewhere[24,25]. Transformation of bacterial cells with DNA was performed using calcium shocked *E. coli* cells as described elsewhere[31].

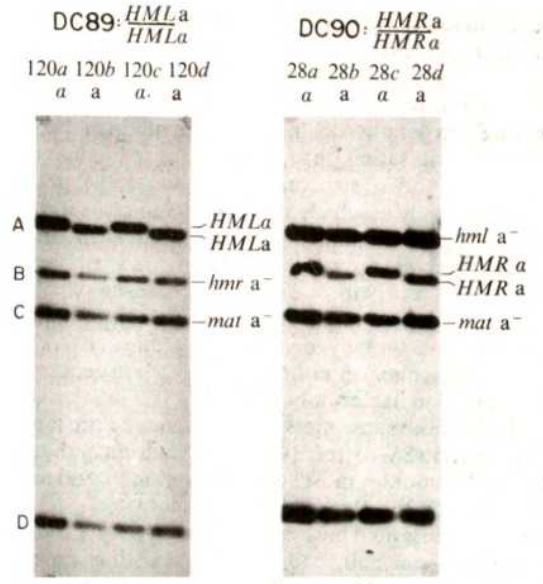


Fig. 4 Autoradiographs of tetrads from diploids DC89 and DC90 showing segregation of size shifts in bands A and B correlated with heterozygosity for *HMLα*/*HML***a** and *HMRα*/*HMR***a**, respectively. DNA was digested with *Hin*dIII and pBR322–26.2 was used as a probe. Approximate sizes of the **a** and α bands in each pair (in kilobase pairs) are: A, 5.8 and 6.0; B, 4.6 and 4.8; C, 4.1 and 4.3.

appears to be ~200 base pairs smaller than the corresponding α (*HMLα*) allele. This size variation segregated in the parental configuration relative to mating type in 12/12 meiotic clones. Similarly, when the *HMR* locus is heterozygous, the size variation can be seen in the second largest band (Fig. 4*b*). Once again, the ~200-base pair larger version co-segregates with the α phenotype, that is, the *HMRα* allele (8/8 meiotic clones tested). The smallest restriction fragment (band D) corresponds to the small partial *Hin*dIII fragment carried on the right-hand end of the *Bam*26 fragment (see Fig. 2) and remains uniform in size.

On the basis of these data, we conclude that the cloned α mating type gene is at least partly homologous with all three loci (*MAT*, *HML*, *HMR*) proposed to contain mating type genes. This homology is observed irrespective of the allele (**a** or α) present at those loci. Furthermore, because a similar size variation is seen at each locus and because such a size difference appears in digests from several different restriction enzymes, we believe that this polymorphism represents an intrinsic size difference between the **a** and α alleles.

Location of the cloned α gene

Because the cloned fragment YEp13-7 hybridises to *MAT*, *HML* and *HMR*, it is not possible from the above observations to determine whether the α gene comes from *MATα* or *HMLα*. Although *HMLα* would not be expected to be expressed in a *MAR*$^+$ strain such as DC49, it is possible that, as an artefact of the cloning procedure, *HMLα* could have been released from the normal mechanism of negative control. In fact, several lines of evidence indicate the α gene identified by function in YEp13-26 is the one originating from *HMLα*: (1) *Bam*H1 fragment 26 (Fig. 2) hybridises more strongly to the restriction fragment identified as *HML* than to *MAT* or *HMR*; (2) the α phenotype of *mar1*$^-$ strains transformed with YEp13-26 is more pronounced than in *MAR*$^+$ strains; and (3) the *Bam*HI-*Eco*RI subclone 26.1 (Fig. 2) does not hybridise to the *Hin*dIII fragments from total yeast DNA identified as containing *MAT* or *HMR* but does hybridise to the *Hin*dIII fragment identified as containing *HML* (data not shown).

Transposition in homothallic switching

From the observations described above, we predicted that the efficient interconversion of mating types observed in homothallic yeast would also involve insertion and excision of DNA at *MAT*. To test this prediction we monitored the effect of a conversion from **a** to α in a homothallic strain. A homothallic (*HO*) haploid normally forms a stable **a**/α diploid cell line through the process of mating type interconversion and mating among the progeny. Once in the **a**/α state, the *HO* switching functions no longer operate and the mating type alleles are stable. However, it is also possible to obtain stable haploid strains containing a functional *HO* gene through manipulation of the states of the *HML* and *HMR* loci. An *HO HMLα MATα HMRα* strain, for example, does not switch and remains an α haploid. The cassette model proposes that such a strain appears stable because it lacks a copy of **a** information. It is therefore possible to construct a strain which has two possible developmental fates. If a cell of genotype *HO HMLα MAT***a** *HMRα* is allowed to divide repeatedly, two cell types are obtained: cells with the original **a** phenotype whose genotype is unchanged, and α cells which now have the *HO HMLα MATα HMRα* genotype. These α cells are no longer capable of switching because they lack a copy of the **a** gene, and, if experimentally isolated from the sibling **a** cells, will grow into a stable α haploid clones. The **a** cells, of course, can still switch, giving rise to addition **a** and α progeny which can mate among themselves to form stable **a**/α diploid cell lines. According to our interpretation of the hybridisation patterns described above, the switching events responsible for the creation of the two distinct cell lines described above should be reflected in the sizes of the restriction fragments carrying mating type genes. The *HO HMLα MATα HMRα* haploid should have only a single *MATα* band, while the diploid *HMLα*/*HMLα MAT***a**/*MATα HMRα*/*HMRα* should contain two *MAT* bands, one derived from the parental **a** allele and one from the newly inserted α allele. Thus we should be able to correlate a change in cell type with a change in the structure of the chromosome in the progeny of a single cell.

The results of such an experiment are shown in Fig. 6. The strains used were created by repeated back crossing (3X) of strains carrying particular arrangements of the *HML* and *HMR* alleles with the normal homothallic diploid X10-1B. X10-1B and JH210 (Fig. 6 lanes *a*, *b*) are stock strains, while lanes *c* and *d* contain DNA from sibling progeny of a single *HO HMLα MAT***a** *HMRα* spore from strain JH204 (Fig. 5). The isolation of these strains followed the strategy described above and used techniques for the pedigree analysis of individual yeast cells described previously[7]. As shown in Fig. 6, the size variation in

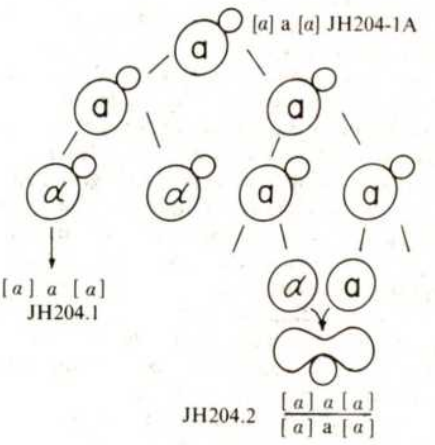


Fig. 5 Schematic of pedigree analysis used to isolate homothallic strains of different developmental potential from a single haploid spore.

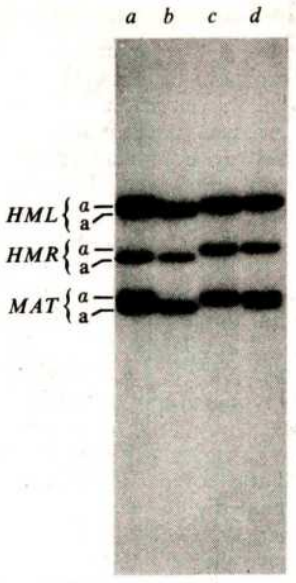


Fig. 6 Autoradiograph of *Hin*dIII digested DNA from related homothallic strains probed with a subfragment of *Bam*26 consisting of the 3.6-kilobase pair *Eco*RI-*Hin*dIII region (see Fig. 1). Lane *a* is the **a**/α diploid X10-1B. Lane *b* is an *HMR***a** haploid strain, JH210. Lanes *c* and *d* represent haploid and diploid derivatives (JH204.1 and JH204.2) of a single haploid spore from JH204.

the *HML*, *HMR* and *MAT* bands follows the genotype of each strain as determined by genetic criteria. Furthermore, the sibling strains (lanes *c* and *d*) which have undergone a homothallic switching event and, thus, a change in developmental potential, show a difference in the banding pattern reflecting the condition of the *MAT* locus. In the haploid α strain (lane *c*) a single *MAT*α band is present, while in the heterozygous diploid (lane *d*) a doublet consisting of one *MAT***a** and one *MAT*α size band appears. It is impossible in such an experiment to trap the original *HO HML*α *MAT***a** *HMR*α cell as a haploid as it continually switches to α and mates. However, we can infer the size of the original *MAT***a** band in that spore from the *MAT***a** bands present in the closely related strains X10-1B and JH210 (lanes *a* and *b*).

Discussion

We have identified and cloned a functional α mating type gene by transformation in yeast. The cloned sequence was identified on the basis of its ability to complement a defect at the *MAT* locus for both mating and sporulation functions. Through subsequent hybridisation analysis we have shown that sequences homologous to the cloned gene exist at three locations in the yeast genome at sites near the *HML*, *HMR* and *MAT* loci. These observations are consistent with the cassette model for mating type interconversion in which the *HML* and *HMR* loci are proposed to contain unexpressed storage copies of the α and **a** mating type genes. The sizes of the three restriction fragments corresponding to these loci depend on the mating type allele present at each locus as defined by genetic criteria. Fragments containing the α allele uniformly appear to be ~200 base pairs larger than corresponding fragments containing **a**. Furthermore, using this apparent size difference, we have shown that both homothallic and heterothallic interconversion of mating types involves a change in size of the restriction fragment mapped to *MAT*. This change in restriction pattern provides a physical demonstration of DNA rearrangement during the interconversion process. We interpret this alteration in restriction pattern as being a consequence of transposition of mating type genes from *HML* and *HMR* to the *MAT* locus.

A fundamental prediction of the cassette model is that the cloned α gene should exhibit strong homology to all copies of α proposed to exist in the genome (*HML*α, *MAT*α, *HMR*α). However, we have observed that this cloned sequence hybridises strongly to genetic loci harbouring **a** mating type alleles as well. This hybridisation may be due to sequence similarity between the **a** and α structural genes or to the presence of recognition sequences which are involved in site specific transposition. We had originally proposed that such recognition sequences might be responsible for the specificity and directionality of the recognition events involved in switching[1,2,32]. However, it is also possible that, although the **a** and α gene products are functionally distinct, they may be coded from closely-related sequences either as overlapping genes similar to those described in bacteriophage ϕX174 (ref. 33) or as processed products of the same message analogous to the large T and small t proteins in SV40 (ref. 34). Determination of the source of the observed homology must await heteroduplex and nucleotide sequence data on the cloned **a** and α genes.

The results presented here confirm the physical predictions of the cassette model and, coupled with existing genetic data, provide strong evidence for transposition of structural genes as a developmental regulatory mechanism. However, the molecular details of the mechanism remain unclear. As the genetic content of the *HML* and *HMR* loci is not altered during switching, it is clear that the transposition event is not reciprocal. Therefore switching must involve both replication of the source gene and substitution of a copy of that gene for the one originally present at *MAT*. It is possible that copies of the source loci are replicated and released as independent molecules which then diffuse to the *MAT* locus and undergo a separate recombination event resulting in substitution. We have looked for a 'diffusible cassette' using Southern blots of undigested DNA from strains constitutive for switching. Such molecules have not been observed in conditions where one molecule per cell would have been easily detectable. Alternatively, replication and substitution need not be separate events but could be the result of a concerted process involving normal recombinatorial intermediates. As we have described elsewhere[32], mating type interconversion is functionally equivalent to unidirectional gene conversion. As substantial homology exists between the source loci and *MAT*, conversion could occur through the same steps proposed for gene conversion at other genetic loci with the proviso that during heteroduplex repair, the source sequences are always maintained. Such unidirectional gene conversion has been observed for certain alleles in both *Sordaria brevicolis*[35] and *Schizosaccharomyces pombe*[36].

The notion that switching might be the result of a modification of normal recombination mechanisms is strengthened by the observation that the *HML*, *HMR* and *MAT* loci are unusually susceptible to intrachromosomal recombination events leading to deletions[37] or chromosomal rearrangements[19]. Genetic selections for rate heterothallic α to **a** interconversions often yield a specific deletion extending from *MAT*α to *HMR***a** (see Fig. 1). This deletion apparently removes the *MAT*α gene and leads to the consitutive expression of *HMR***a**. The converse fusion of *MAT***a** to *HML*α yields a circular chromosome containing the centromere in which *MAT***a** has been removed and *HML*α is expressed[19]. These events may be due to pairing of homologous sequences followed by generalised reciprocal recombination or may represent abortive resolution of intermediates generated during site-specific transposition.

Although our results show that switching from **a** to α involves the net addition of a ~200-base pair segment to the *MAT* restriction fragment, the actual size of the transposed region has not been determined. One possible interpretation of this result is that the expression of the α and **a** mating type genes at *MAT*, *HML* or *HMR* depends solely on the presence or absence, respectively, of a 200-base pair translocatable element. Recent genetic results, however, make this possibility unlikely. Klar and Fogel[21] and Kushner *et al.*[20] have shown that mutant information at the *HML* and *HMR* loci can be transposed to *MAT*

during both homothallic and heterothallic switching. Therefore some sequences present in the a and α structural genes must be transposed during switching. Our results further indicate that the α transposon is less than 3.6 kilobase pairs as described in Fig. 2 legend.

We have demonstrated that interconversion of mating types in *Saccharomyces* involves an alteration of the DNA sequence at the mating type locus of this organism. We believe that this genetic change is the result of sequential transposition of a and α structural genes to *MAT* from the *HML* and *HMR* storage loci. This change is heritable but is also subject to subsequent transposition events. These interconversions of cell type occur in an orderly fashion: (1) they occur in a defined subset of clonally-related cells[3,7]; and (2) cells do not randomly become a or α; they are directed to change to the new cell type by functions under the control of mating type alleles[3,7,14]. Thus, this simple mating type switch exhibits many of the properties observed in the differentiation of cell types in more complex organisms and the mechanism of mating type interconversion provides a paradigm for the study of other developmental events.

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letters

A radio jet in SS433

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The peculiar emission line object SS433 (refs 1–3) has variable radio emission and an angular size of <0.1 arc s at centimetric wavelengths[4,5]. The observations reported here show that the object at long radio wavelengths consists of this compact core and also a jet ~1 arc s long aligned in the same position angle as the extended structure in the associated supernova remnant W50. Both components vary in flux density.

The observations were made at 408 MHz with radio-linked interferometers consisting of the MK IA 76 m or the MK II 25 m × 37 m telescopes at Jodrell Bank and the 25 m telescope at Defford. All telescopes were equipped with 1-h circularly polarised feeds. The baseline length was 127 km, giving a resolution limit of 0.2 arc s. The dates of the observations are shown in Table 1. The source was tracked for long periods at all except the first two epochs, enabling models of the radio structure to be fitted to the fringe amplitudes obtained. Short observations only were available on the first two dates, but these were sufficient to allow the flux densities of an unresolved core and an extended component to be found.

A sample visibility curve (that of 10 August 1979) is shown in Fig. 1. Each point represents the average of four integrations of 30 s duration. Also shown is the predicted visibility from a model consisting of an unresolved core (<0.2 arc s diameter) with a flux density of 0.8 Jy plus a gaussian component of 0.9 Jy with dimensions of 1.0 arc s FWHM by <0.2 arc s and elongated in position angle 100°. Similar models were fitted on the other dates giving flux densities, lengths of the elongated component and position angles as shown in Table 1. The flux densities of the two components show significant variations while changes in size and position angle are within measurement errors. The range of resolution available was not sufficient to establish whether the jet is symmetric about the core or displaced by up to ~0.5 arc s in position angle 100°. In addition, lack of phase information gives rise to an ambiguity of 180° in position angle. Nevertheless, the effect of an elongated component is quite clearly seen in the data.

This jet-like feature is very similar to structures found in the central regions of some extragalactic radio sources such as 3C236 (ref. 6) and NGC6251 (ref. 7). It has been proposed that these regions are the sites of radio emission from beams of relativistic matter flowing out from a central nucleus. Such beams may therefore be present in SS433 though at a much lower power level and smaller linear scale. Indeed, beams have

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Table 1 Details of observations on SS433

Instrument	Date	Total flux density (Jy)	Core flux density (Jy)	Extended component flux density (Jy)	Size (arc s)	Position angle
MK IA-Defford	27 May 1976	1.9±0.3	1.3±0.3	0.6±0.4	—	—
MK IA-Defford	4 February 1979	1.5±0.2	1.3±0.2	0.2±0.2	—	—
MK IA-Defford	6 June 1979	2.6±0.2	2.2±0.2	0.4±0.2	1.5	99°
MK II-Defford	31 July 1979	1.1±0.1	0.5±0.1	0.6±0.1	1.0	102°
MK II-Defford	9 August 1979	1.7±0.1	0.9±0.1	0.8±0.1	1.2	98°
MK II-Defford	10 August 1979	1.7±0.1	0.8±0.1	0.9±0.1	1.0	100°
MK II-Defford	11 August 1979	1.7±0.1	0.8±0.1	0.9±0.1	0.9	97°
MK II-Defford	12 August 1979	1.8±0.1	0.8±0.1	1.0±0.1	0.8	97°

been proposed[8,9] to explain the moving optical emission lines in SS433 and Begelman *et al.*[10] have further suggested that these beams supply energy to the supernova remnant W50. On single-dish maps[11,12] W50 has the appearance of a filled spherical shell of emission ~1° in diameter with regions extending to ~2° in overall size in position angle 100±10°. The orientation of the jet found here (99°) suggests that these outer regions have been blown out by beams from SS433. The range of angular scales involved is ~7,000:1 and the beams must have maintained their orientation within 10° for at least the light travel time from SS433 to the outer regions of W50 which is ~200 yr if SS433 is the suggested distance of 3.5 kpc (ref. 3).

The moving optical emission shows a periodicity in velocity of 164 days and it has been proposed that beams responsible for the emission describe a cone of half-opening angle 17° inclined at 78° to the line of sight[9]. Such motion may be caused by precession of the parent object[10]. The phase of the precession is such that the position angle would be expected to change by 30° from 6 June to 12 August 1979, whereas the measurements show that any precession of the radio jet must be ≲5° over this period. The length of the radio jet if SS433 is at a distance of 3.5 kpc is 5×10^{16} cm, about 100 times the size suggested for the moving regions of optical emission [10] and so it is possible that the effects of precession on position angle are smoothed out within the radio jet.

The flux densities of both the core and jet varied, particularly in the interval between 6 June 1979 and 31 July 1979. The electron lifetime at 1 GHz, if equipartition between relativistic electron energy density and magnetic energy density is assumed in the jet, is ~1,000 yr and so the variability is more likely to arise from adiabatic expansion of small emitting regions in the jet than from radiative energy losses. The core variability may be associated with a changing angle between the beams and the line of sight as recently discussed for much more powerful radio sources[13]. Further study of the flux density variations of the core and jet over several periods and high-resolution VLBI maps will show if this is the case, and give information on relationships between the optical properties and the radio structure.

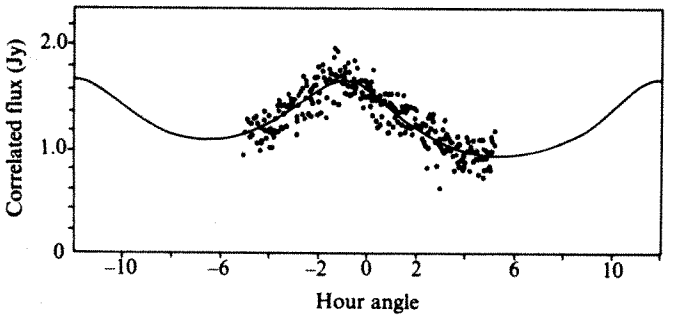


Fig. 1 Fringe amplitude versus interferometer hour angle on 10 August 1979. The smooth curve shows the expected fringe amplitude from a model consisting of a point source coincident with an extended gaussian component 1.0 arc s long to half maximum brightness and <0.2 arc s wide, with flux densities as given in Table 1.

I thank Dr Dennis Walsh for data and Owen Stewart for assistance.

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Low energy γ-ray spectrum of NGC4151

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Following the first detection of X-ray emission from the Seyfert galaxy NGC4151[1], later observations[2–4] have demonstrated that the X-ray spectrum up to 100 keV may be described by a power law having a differential spectral index $1<\alpha<1.6$ with a turnover at low energies caused by photoelectric absorption. Recently the results of an observation of this galaxy in the energy range 25–190 keV (ref. 5) have shown it to have a very hard spectrum with $\alpha\cong0.9$. Here more detailed results of the original observation of NGC4151 (ref. 6) using the MISO telescope are presented.

The Seyfert galaxy NGC4151 was studied during the first balloon flight of the MISO telescope[7] on the 22 May 1977. The instrument consisted of a Compton coincidence detector having in this flight an aperture of 3°×20° FWHM which was the same for both the 'single' and 'coincidence' γ-ray events. The telescope (energy range 0.04–20 MeV) was pointed using an alt–azimuth orientation system which had a precision of about 0.3°.

NGC4151 was observed at two different zenith angles (18° and 7°) over a period of ~3 h. Half of this time was devoted to

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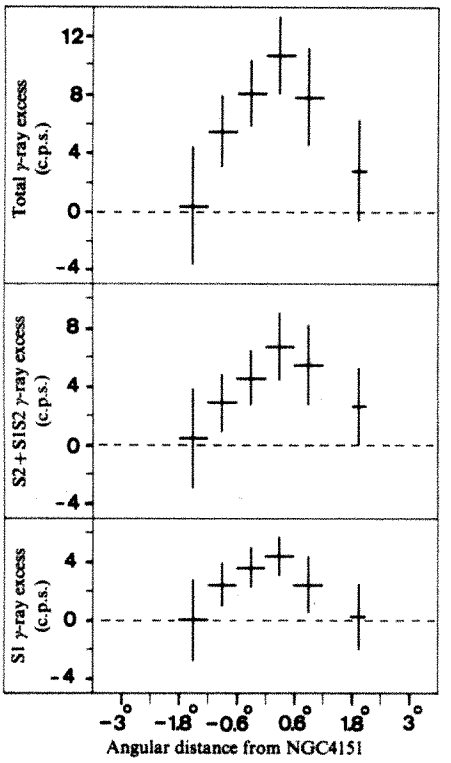


Fig. 1 γ-Ray excesses count rate (S1, S2 + S1.S2, total) versus the angle between the azimuthal axis of the MISO telescope collimator and the direction of NGC4151. The background measurement was made in a region of the sky some way (> 10°) from NGC4151.

background measurements. Observations at each zenith angle were made using an off–on–off source sequence. The source was allowed to transit through the field of view of the telescope and care was taken to ensure that no known X-and γ-ray sources were in the field of view during the background measurements. During the two observations of NGC4151 the float altitude varied between 6.2 and 8.0 mb but stayed close to 7 mb during the ON source observation. The geographical position remained practically constant during the entire observation period. To eliminate any systematic errors due to zenith variations of the background, the data were analysed separately for each zenith angle and the results statistically combined. No dependence of the counting rate on the azimuth angle was found. This means that, if an azimuthal effect exists, it is much less than the statistical errors, as expected from the fact that the entire observation was carried out at small zenith angles. Study of the housekeeping data and in flight γ-ray calibrations showed that there were no measurable changes in the gain of the system during the period. The only measurable systematic change of the background counting rate was due to altitude fluctuations. This variation, measured during the OFF source period, was found to be well described by a linear relationship with pressure for each detector element at both zenith angles. During the ON source period the background counting rate, computed from a knowledge of the balloon altitude, was subtracted from the counting rate generated by the source plus background for each channel. In this way the contribution from the source was obtained, giving a γ-ray excess of 3.0 ± 0.7 counts per second (c.p.s.) for the S1 detector (liquid scintillator), 3.1 ± 0.9 c.p.s. for the S2 detector (sodium iodide) and 1.1 ± 0.4 (c.p.s.) for the S1S2 Compton-coincidence system. The total γ-ray excess was 7.2 ± 1.2 c.p.s., which corresponded to a 6σ excess. This was considerably larger than the upper limit of the sum of all possible systematic errors. The correlation between the excess count rate and the angle between the azimuthal axis of the collimator and the direction of NGC4151 is shown in Fig. 1. The absence of data between 1.2° and 1.8° in Fig. 1 is associated with a loss of recorded data. The lack of data just outside the 3° field of view of the MISO telescope is a consequence of the background measurements being made in a region of the sky some way (> 10°) from NGC4151. A χ^2 best fit of the angular profile data gives the γ-ray excess emission centred about a position $182.2° \pm 0.8°$ in RA, which effectively coincides with the position of the Seyfert galaxy NGC4151 (RA = 182.06°). The positional uncertainty associated with the second (zenith) axis is about ± 10°. The only other known X-ray source which came within the field of view during this observation was the BL Lac object 2A1219+305. However, as this object was at the edge of the aperture of the telescope (10% relative efficiency for photon detection) the proposed identification of the observed γ-ray excess with NGC4151 seems justified.

A technique similar to that used by Dolan[8] was used to convert the energy loss spectrum in each of the three individual γ-ray channels S1, S2, S1S2 to a photon spectrum at the top of atmosphere. The absorption of photons in the residual atmosphere and the redistribution of photon energies through Compton interactions and pair production as well as the energy resolution of the telescope were taken into account. The precision of the system was estimated to be better than 10%. A statistical combination of all three individual spectra is shown in Fig. 2 together with some relevant X-ray measurements. For clarity, the measurements of OSO7 (ref. 2) and the 1976 Ariel 5

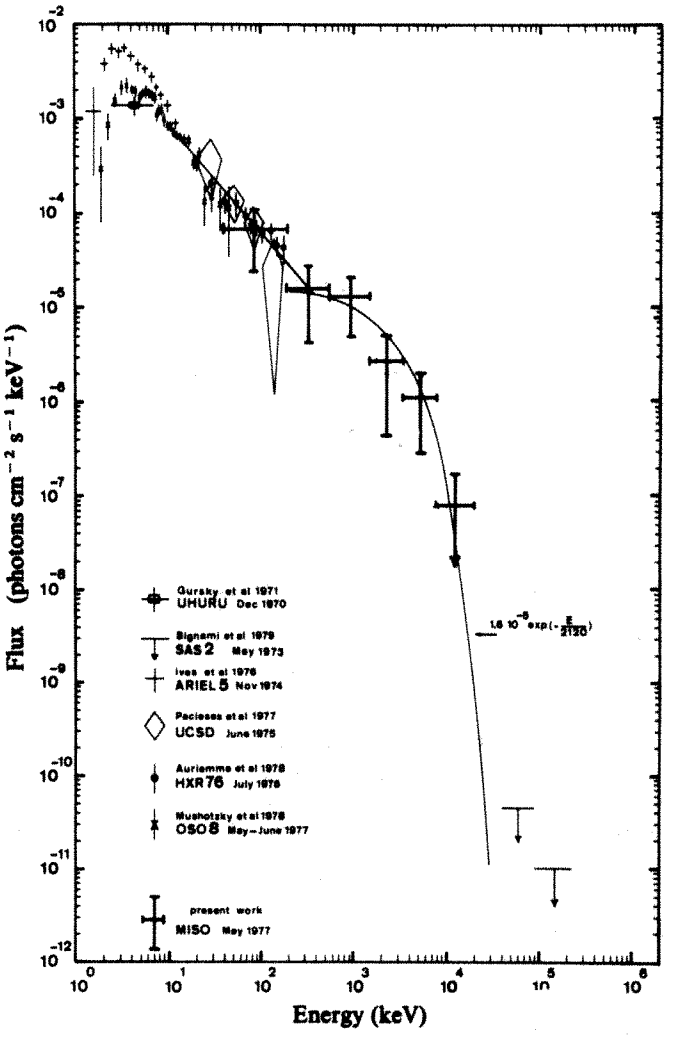


Fig. 2 The photon spectrum of NGC4151 obtained by the MISO telescope is shown together with some previous hard X-ray data. Also shown are the 2σ upper limits obtained by SAS2 at high energy[11].

data[9] have not been shown as they are essentially identical to the more recent measurements of OSO8 (ref. 10). The figure does not show the low energy γ-ray results reported earlier by the Max Planck group[11] because of the uncertainty cast on the validity of this measurement by a more refined analysis of the data[12]. Also shown are the 2σ upper limits set by SAS2 at higher energies[13].

The Ariel 5 (ref. 14) and the OSO8 data provide evidence for the variability of NGC4151 on a time scale of about one week with changes in the flux of about a factor of 2. The observation with the MISO telescope was made on the 23 May 1977, a few days before the OSO8 observations. Inspection of the light curve from OSO8 suggests that the source was in 'high' state of activity during our observation period. The recently reported upper limits at MeV energies by the Rice group[15] and by the University of California[16] indicate that time variability in the γ-ray emission of NGC4151 must exist over a period of several months.

An attempt to fit our data with a single power law leads to a reduced $\chi^2 \cong 1.9$ with 4 d.f. The variability of this source is well established and the use of data taken at different epochs is not entirely satisfactory, however, when X-ray data above 20 keV (refs 4, 5, 10) are included, a similar treatment over the entire energy range leads to a reduced $\chi^2 \cong 2.3$ with 19 d.f. On the other hand, the data can be fitted better with a power law ($\alpha \cong 0.9$) up to 2.5–4 MeV followed by a break and a steeper spectrum ($\alpha = 3.4$–4) at higher energies as suggested by both our data and the upper limits set by SAS2. The resulting reduced χ^2 value for this kind of fit is 0.73 with 19 d.f. Alternatively, our measurements may be represented at energies higher than 200 keV by an exponential of the form

$$dI/dE = (1.6 \pm 0.7)10^{-5} \exp(-E/(2120 \pm 480))$$

photons $cm^{-2}\,s^{-1}\,keV^{-1}$

corresponding to a reduced $\chi^2 \cong 0.5$ with 3 d.f. The data between 10 and 200 keV would then be best fit by a power law of the form

$$dI/dE = (1.1 \pm 0.3)10^{-2}\, E^{-(1.1 \pm 0.1)}$$

photons $cm^{-2}\,s^{-1}\,keV^{-1}$

corresponding to a reduced $\chi^2 \cong 1.1$ with 23 d.f. These two fits are shown in Fig. 2. During our observation, the power output in the range 0.5–5.0 MeV was the highest observed for NGC4151 ($\sim 2 \times 10^{45}$ erg s^{-1} for a distance of 20 Mpc, $H_0 = 50$ km s^{-1} Mpc^{-1}). In the adjacent decades, 50–500 keV and 5–50 MeV, the power was $\sim 2 \times 10^{44}$ and $\sim 6 \times 10^{44}$ erg s^{-1} respectively.

Several models have been proposed to explain the emission from active galaxies up to γ-ray energies. The suggested mechanisms are the inverse Compton scattering of the synchrotron radiation photons[17–20], the accretion onto massive black holes[21–23] or the rotation of magnetised objects (spinar model)[24]. For the non-thermal models, the e-folding energy of the exponential fit (or the cutoff of the power fit) may arise from an equivalent cut-off in the electron spectrum that produces the γ-ray emission or may be due to the opacity of the source for pair production. For a thermal model interpretation, our data suggest a temperature of about 10^{10} K in the active region of the galaxy. An alternative may be the "Penrose Compton Scattering" model proposed by Leiter and Kafatos[25] in the hypothesis of the existence of a massive Kerr black hole at the centre of active galaxies. It is clear that models which predict a cutoff around 500 keV (see ref. 22) are not favoured by our spectral data.

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Evidence for low-energy γ-ray emission close to CG135+1

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The COS-B source CG135+1 (ref. 1) is an extremely interesting object because of its possible identification with the QSO 0241+622. Radio observations have identified a strong point source within 2 arc s (ref. 2) of QSO 0241+622. X-ray emissions have also been observed from this region of the sky by the Uhuru (ref. 3), SAS-3 (ref. 2) and HEAO-A (ref. 4) telescopes. The HEAO-A (A1) data indicate that there are two soft X-ray sources in this region of the sky, H0241+62 which contains the QSO, and H0235+60 which includes the radio source GT0236+61 (ref. 5). The OSO-7 data in the energy range 1–40 keV (ref. 6) and measurements using the high-energy X-ray experiment on Ariel-V between 0.26 and 1.2 MeV (ref. 7) have indicated that the photon emission from this region of the sky is of an extremely hard nature, with a power law spectral index of $\gamma \approx 1.0$. However, the poor angular resolution of these telescopes make it uncertain whether the high-energy X-ray fluxes are due to the same celestial object. We now report the results of a balloon flight carried out on 8 October 1978 from Palestine, Texas, in which the region of the sky including the COS-B source CG135+1 was scanned by the Milan/Southampton telescope (MISO telescope). A 5σ excess in the counting rate of the telescope in the energy range 0.15–20 MeV was observed. The origin of this low-energy γ-ray emission is compatible with the measured position of CG135+1.

The MISO telescope used to study this region of the sky in the low-energy γ-ray region of the spectrum has been described elsewhere[8] and consists of two scintillators, S1 (liquid scintillator) and S2 (sodium iodide), which form the central Compton coincidence detection system. The instrument was sensitive over the energy range 0.15–20 MeV and had an aperture of 3° FWHM in both azimuth and zenith planes for this observation. The telescope was stabilised in both the azimuth and zenith planes with a precision of 20 arc min by means of a two-axis orientation system.

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Six drift scans were carried out to survey the region of the sky contained within the coordinate points 2 h 16 min and 3 h 12 min in right ascension and centred on 62° in declination, over the period 6–10 h in universal time. To obtain a reasonable number of scans of the source region during the 4-h observational period available, the telescope was driven in azimuth against the rotation of the sky with a speed of approximately 0.2 deg min^{-1}. The region of the celestial sphere studied encompasses the error boxes of the X- and γ-ray emissions measured by the Uhuru, HEAO-A, SAS-3, OSO-7, Ariel-V and COS-B telescopes. The measurements were made at a mean residual atmospheric pressure of 3.6 mbar with the telescope set at zenith angles in the range 30–34°. The results reported here refer to a preliminary analysis of the integral γ-ray counting rate above 150 KeV, for five passes. Each of the five drift scan measurements was analysed separately so as to eliminate effectively any systematic variations due to zenith and azimuth changes of the background counting rates. The housekeeping data and the spectra from γ-ray calibration sources were studied to ensure that there were no overall measurable changes in the gain of the system during this period. The only source of measurable systematic variation in the background counting rate was due to the change of altitude between 3.5 and 3.9 mbar, and the data have been corrected for this effect.

The background counting rate as a function of pressure was evaluated separately for each drift scan and for each of the three independent counting rate channels (S1, S2, S1.S2). After subtraction of the background a correlation was made between the residual counting rate and the angular distance between the axis of the telescope and the direction of CG135+1. For each pass there was an indication of an excess from the general direction of CG135+1. The results were combined statistically and are shown in Fig. 1. An excess is observed for each of the three independent channels (S1.S2 = 0.65 ± 0.2 c.p.s., S1 = 1.3 ± 0.5 c.p.s., S2 = 2.1 ± 0.6 c.p.s.). The statistical errors quoted here include the uncertainty in the evaluation of the variation of counting rate with residual pressure. Figure 1 also shows the data for the sum of all three channels in which an excess of 5σ is obtained (4.1 ± 0.8 c.p.s.). The solid line in Fig. 1 represents the exposure of the centre of the COS-B error box defined by the aperture of the MISO telescope (3° FWHM). The angular profiles of the excesses can be seen to follow closely the aperture of the MISO telescope.

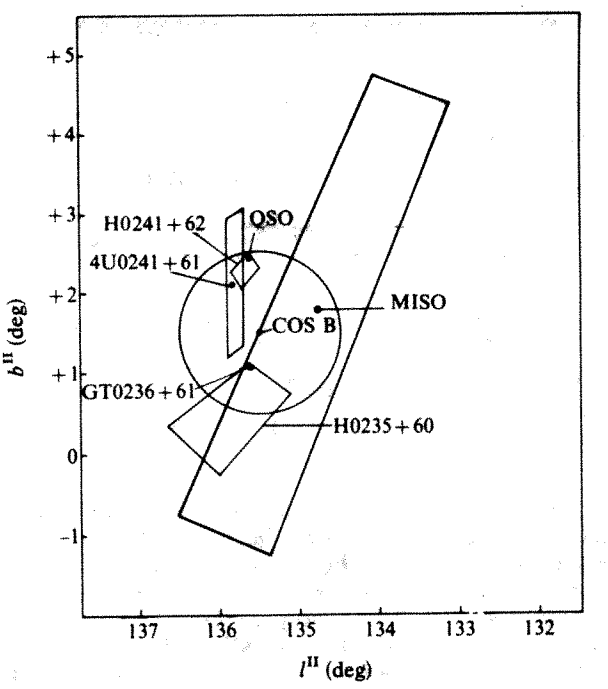


Fig. 2 The MISO error box in galactic coordinates (l^{II} ≡ longitude; b^{II} ≡ latitude) is shown together with other astronomical data. The MISO error box has 68% probability across the scan direction and 100% in the other.

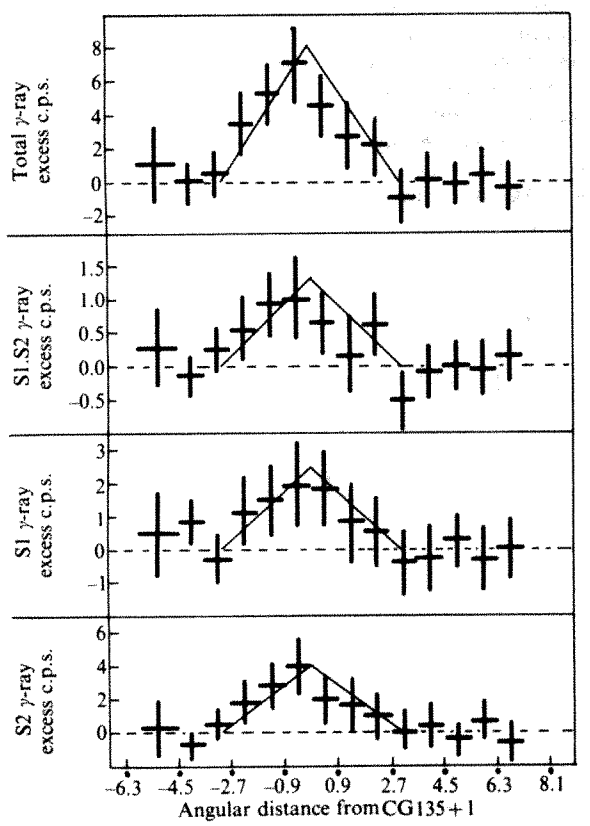


Fig. 1 The γ-ray excess plotted against the angular distance from CG135+1 is shown for the 'singles', the coincidence and the total events. The solid line represents the exposure of the centre of the COS-B error-box defined by the aperture of the MISO telescope (3° FWHM).

Although the data indicate a 0.6° difference between the centroids of the MISO and COS-B emissions, the results are compatible with a common celestial origin. Figure 2 shows the MISO error box in galactic coordinates for the observed γ-ray flux together with other astronomical data taken at different photon energies. As our drift scans were made in one direction only, the limits of our error box are represented by the 1σ points along the direction of scan and by the total aperture of the telescope in the orthogonal direction (100% probability).

If it is assumed that the X-ray and COS-B γ-ray flux measurements originate from the same source object, a steepening of the spectrum is required above 1 MeV. On the basis of our integral counting rate of 4.1 ± 0.8 c.p.s. the spectral change must be close to 1 MeV. Only the full spectral analysis of our data will enable us to say more on the existence of such a change and consequently on the coincidence of the X- and γ-ray emission.

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Separation of ^{26}Al and ^{26}Mg isobars by negative ion mass spectrometry

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Aluminium-26 (half life 7.16×10^5 yr) is one of the important radioisotopes generated from the spallation by cosmic rays of argon in the Earth's atmosphere. It has been used in studies[1] of the growth rate of manganese nodules from the ocean floor and the constancy of the cosmic-ray intensity in the past[2]. At present, the concentration of ^{26}Al ($^{26}Al/Al\leq10^{-14}$) is measured in large samples by counting the γ rays following the β decay to ^{26}Mg. We have now demonstrated that it is possible to separate ^{26}Al and ^{26}Mg atoms in milligramme samples with a sensitivity for the ratio $^{26}Al/Al$ better than 10^{-14}, using negative ions, a tandem accelerator as a molecular disintegrator, and a mass spectrometer[3]. The measurement of $^{26}Al/^{10}Be$ ratios using the same apparatus and small samples, to provide a several million year chronology[4,5] for oceanic sediments, now seems possible.

The complete separation of the isobars ^{26}Mg and ^{26}Al using negative ion mass spectrometry was shown to be possible in principle by Heinemeier and Hvelplund[6] during measurements on the production of Mg^- and Al^- in sodium vapour. They observed that for 20 keV ions at equilibrium in sodium vapour the ratio of Al^-/Al^+ was 0.10 whereas the ratio of Mg^-/Mg^+ was less than 10^{-6}, implying a highly unstable magnesium negative ion.

Soon after the first detection by atom counting of ^{14}C (half life 5,730 yr) at natural abundances[7], we tried unsuccessfully to use negative ions to detect ^{26}Al at levels of $^{26}Al/Al$ near 10^{-12}. It was realised during several of the early experiments that the problem was due to background from molecular fragments from ions such as $^{12}C^{14}N^-$, and from ^{27}Al positive ions from the injection of $^{27}Al^-$ ions. The abundant ^{27}Al positive ions were present because the mass resolution of the magnet at the ion source was not high enough to reject $^{27}Al^-$ ions when $^{26}Al^-$ ions were injected into the tandem accelerator. These problems can be solved by the addition of an electrostatic analyser, which specifies the ratio of the ion energy and the charge (E/q), to the magnetic analyser after the accelerator which specifies $(M/q)\times(E/q)$. M is the mass of the ions. The use of the combination of electrostatic and magnetic analysis is most successful when M and q do not contain common factors[8].

After the installation of a 10° electrostatic analyser, successful measurements of $^{36}Cl/Cl$ ratios down to 10^{-15} (^{36}Cl half life 3.08×10^5 yr) were made and reported[8]. The apparatus has since been used[9] to measure $^{10}Be/Be$ ratios to below 10^{-14} (^{10}Be half life 1.6×10^6 yr) and $^{36}Cl/Cl$ ratios in meteoritic and other samples[10] to near 10^{-16}. The same apparatus[8] has now been used to detect ^{26}Al. Recently, Raisbeck *et al.*[11] have reported the detection of ^{26}Al by accelerating positive ions to a high energy in a cyclotron and removing all the electrons so that $^{26}Al^{13+}$ and $^{26}Mg^{12+}$ can be completely separated in a magnetic field. A sensitivity for the ratio $^{26}Al/Al$ of 10^{-12} was reached. The complete stripping of the ions solved the problem[12] of detecting ^{26}Al with a cyclotron.

Two samples of aluminium and one of magnesium were prepared for use in the negative ion sputter source of the University of Rochester MP tandem Van de Graaff accelerator. The first sample, of 99.999% pure aluminium, was used for background measurements and the second was machined from an aluminium–magnesium (1%) alloy which had been exposed some years ago to high energy electrons and photons in the beam dump of the 40 MeV Electron Linac at Toronto. The $^{26}Al/Al$ ratio was estimated to be less than 2×10^{-11} by measurements of the γ rays following the β decay of ^{26}Al in a 10-g sample. The magnesium sample was used to increase the sensitivity of the detection system for $^{26}Mg^-$ ions.

The negative ion current of $^{27}Al^-$ from the ion source was about 15 nA during the measurements. This is a low value compared with the current of 1 μA which has been reported from a negative ion sputter source (R. Middleton, personal communication) but it was found to be adequate for the demonstration of the separation of ^{26}Al and ^{26}Mg by negative ion mass spectrometry.

The negative ^{26}Al ions were accelerated to a total energy of 48.17 MeV in the tandem accelerator and the $^{26}Al^{5+}$ ions were analysed by the equipment used previously for ^{36}Cl detection. The dissociation of the molecular ions after charge exchange in the tandem accelerator and the electrostatic analysis provide an efficient way of eliminating such mass 26 molecules as $^{13}C_2$, $^{10}B^{16}O$, and $^{12}C^{14}N$ without the need for high mass resolution. Figure 1 illustrates in a two-parameter plot the clear separation between $^{26}Al^{5+}$ and $^{26}Mg^{5+}$ from the irradiated sample even though there was 1% magnesium in the sample. The measured $^{26}Al^{5+}$ counting rate of (1.5 ± 0.3) c.p.m. and the average analysed $^{27}Al^{5+}$ ion current of 5.3 nA implies a ratio for $^{26}Al/Al$ of $(3.8\pm0.7)\times10^{-12}$ in the irradiated sample. During experiments with the unirradiated pure aluminium sample, no ^{26}Al counts were recorded in a total of 230 min; one count would have implied that an $^{26}Al/Al$ ratio of 10^{-14} was reached. To guard against drifting of the magnets, ion source fluctuations, and other systematic effects, the irradiated aluminium sample was counted immediately before and immediately after the background run on the pure Al sample. The counting rate of the irradiated sample was the same within statistical error for both of these runs, indicating that the system parameters had not changed significantly during the 230 min.

An estimate of the mass of aluminium sputtered during the experiment (1.39 mg) yielded an efficiency for producing negative ions Al^-/Al of about 10^{-3} and a total efficiency for detecting

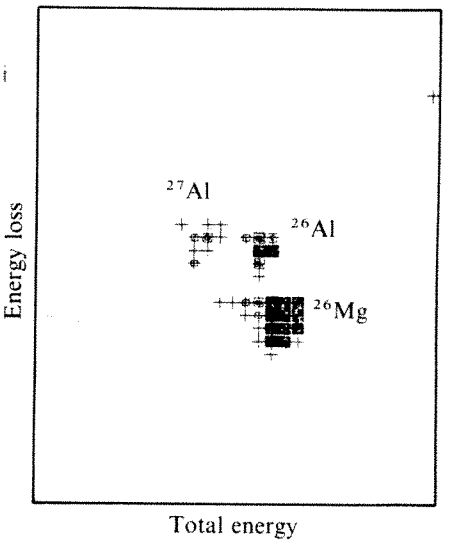


Fig. 1 Counts versus energy loss and total energy from an ionisation counter for an aluminium–magnesium (1%) alloy enriched in ^{26}Al. The few ^{27}Al counts detected at 46.4 MeV are some of the ions from multiple charge exchange in the accelerator tube which gives the required magnetic rigidity. A total of 34 counts corresponding to 48.17 MeV $^{26}Al^{5+}$ and 1,250 counts for $^{26}Mg^{5+}$ were recorded in 23 min. The ^{26}Mg contribution could be reduced substantially by chemical processing of natural samples. No ^{26}Al counts were observed from a pure aluminium sample.

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$^{26}Al^{5+}/Al$ from the sample of about 10^{-4}. Even this low efficiency implies that the negative ion method is already superior to the γ-ray counting method for determining $^{26}Al/Al$ ratios below 10^{-12} in samples smaller than about 1 g.

The small current of Al^- ions used for these experiments can be increased by using a more powerful ion source. The efficiency for utilisation of the ^{26}Al atoms and the Al^- current can also be increased by starting with positive sputtered ions and charge exchanging in sodium vapour[6]. In either case currents over 1 μA should make possible measurements of $^{26}Al/Al$ ratios below 10^{-15} in under an hour from samples of aluminium possibly as small as 1 mg. This amount of aluminium can be found in as little as 10 mg of oceanic sediment[4]. The large tandem accelerator used for these demonstration experiments can also, in principle, be replaced by a much smaller tandem accelerator such as those at present under construction specifically for ^{14}C dating[13] as the difference in energy loss (dE/dx) for ^{26}Al and ^{26}Mg, 10.7% in the present experiment, changes to only 7.1% for the smaller machine.

In Fig. 1, a peak corresponding to $^{26}Mg^{5+}$ can also be seen. It was demonstrated that the flux of $^{26}Mg^{5+}$ ions clearly peaked at the terminal voltage used in these measurements and also reached another much larger peak when the tandem terminal voltage was raised 58 ± 8 kV. These $^{26}Mg^{5+}$ ions from the larger peak are from the injection of $^{26}MgH^-$ molecular ions into the accelerator due to the inadequate resolution of the injection magnet ($M/\Delta M \sim 25$) or the breakup of $^{26}MgH_2^-$ into $^{26}MgH^-$ before magnetic analysis. The addition of an electrostatic analyser at the ion source would remove the second source of background. It is worth noting here that the aluminium alloy sample, containing 1% magnesium, enhances the yield of magnesium ions over that of a pure sample.

The $^{26}Mg^{5+}$ ions that appear at the same terminal voltage as the $^{26}Al^{5+}$ ions could be due to the injection of $^{26}Mg^-$ into the accelerator. This, we believe, is an unlikely explanation as the very low limit[6] for the formation of Mg^- can best be explained by a mean lifetime for Mg^- of less than about 10^{-7} s. As the acceleration time for the negative ions in the present experiment is about 10^{-5} s, no Mg^- ions should survive the acceleration. In addition, the efficiency for formation of such metastable ions in a negative sputter ion source is expected to be very low. (R. Middleton, personal communication). The absence of Mg^- ions in the observations of Heinemeier and Hvelplund[6] could also be due to the low binding energy of the electron in Mg^- and to the destruction[14] of Mg^- in the electric analysing field. If this occurs, it can be estimated[14] that the electron binding energy of the negative ion would have to be less than 1 meV. A Mg^- ion with such a low binding energy would be destroyed completely in the high electric fields of the tandem accelerator. These fragile $^{26}Mg^-$ ions could also contribute to the observed $^{26}Mg^{5+}$ ions as a result of multiple charge exchange processes. Further research into the origin of this $^{26}Mg^{5+}$ peak is desirable although it is not expected to interfere with the detection of ^{26}Al in aluminium provided the magnesium abundance is less than 1%.

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A maximum speed of wetting

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The wetting (or dewetting) of a solid by a liquid is an integral part of many important processes such as coating, petroleum recovery, distillation and the handling of liquid fuels in low gravity conditions. Several experiments[1–4] have shown that wetting lines (where liquid, air and solid phases meet) which are straight at slow rates of movement over the solid have a sawtooth shape at sufficiently high speeds. We now offer a quantitative explanation for this phenomenon based on the postulate that, for a given system, there is a maximum rate at which wetting can proceed. The consequences of this interpretation are likely to be important, since, in many practical situations, the aim is to maximise the speed of wetting without entraining the displaced phase.

In our experiments, a tape plunges vertically into a pool of liquid. We measure the dynamic contact angle (the angle that the liquid/air interface makes with the moving tape), and we observe the behaviour of the wetting line. At low tape speeds, the wetting line is straight and horizontal. As the speed is increased, the wetting line is pulled below the level of the free liquid surface and the advancing contact angle measured through the liquid increases, reaching 180° at some critical speed U. At tape speeds exceeding U, the wetting line breaks into two or more straight-line segments inclined to the horizontal at an angle ϕ which increases steadily with speed. In other words, the wetting line adopts a sawtooth configuration (Fig. 1). At sufficiently high speeds, air bubbles break off the trailing (deeper) vertices and are entrained into the liquid.

Wetting has been modelled[5] as an adsorption/desorption process in which, as the wetting line advances, molecules adsorbed at the initial solid/fluid interface are randomly but steadily displaced by molecules from the advancing fluid and a new, more or less equilibrium solid/fluid interface is created. This model has been analysed[5,6] using the statistical mechanical theory of transport processes developed by Eyring and co-workers[7], and an expression has been derived relating the dynamic contact angle to the speed of wetting. The form of the

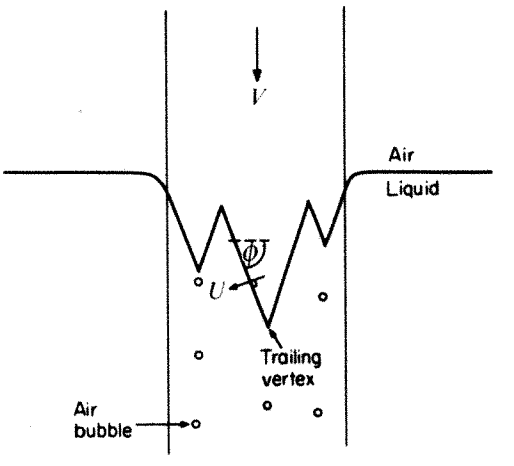


Fig. 1 Schematic representation of the sawtooth wetting line obtained when a tape plunges vertically into a pool of liquid at a speed, V, greater than the maximum speed of wetting, U. The wetting line shows both leading and trailing vertices, with air bubbles entrained at the latter.

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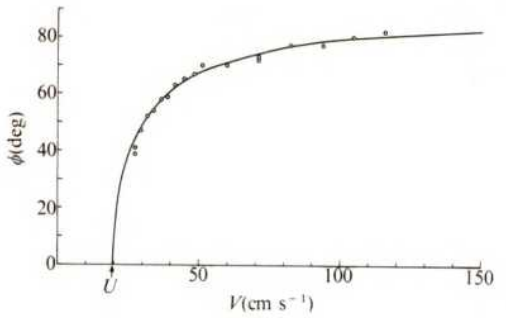


Fig. 2 Measured values of ϕ, the angle of inclination of the wetting line with respect to the horizontal, for a polyester tape (Kodak Estar film base) 5 cm in width and 0.018 cm in thickness plunging at speed V into a nominally 85.6% glycerol/water mixture (viscosity 1.0 P, surface tension 66 dyn cm^{-1}). The theoretical curve was fitted to the data to determine the maximum speed of wetting, $U = 19.4$ cm s^{-1}.

relationship leads to the interesting prediction that there is a maximum speed at which a wetting line can advance normal to itself, that is, a maximum rate of wetting per unit length of wetting line (area/time). This maximum corresponds to a dynamic advancing contact angle of 180°. Conversely, there is a maximum rate of dewetting corresponding to a dynamic receding contact angle of 0°. According to the argument set out below, the breakup of the wetting line into segments slanted from the horizontal is evidence of these speed limitations.

If we suppose that there is a maximum speed, U, for wetting corresponding to a dynamic contact angle of 180°, and if we further suppose that it is the component of tape velocity V normal to the wetting line which is relevant to the wetting process and not the tape velocity itself, then it follows that the tape can be wet at speeds exceeding U only if the wetting line slants from the horizontal at an angle, ϵ, which ensures that the component of tape velocity normal to the wetting line does not exceed U. That is, it follows that

$$\cos \quad \leqslant U/V; \qquad (V \geqslant U)$$

To test this relationship, we have measured as a function of speed for a polyester tape plunging vertically into an aqueous glycerol solution. The data are shown in Fig. 2. The curve through the data is the limit of the above inequality, and so it appears that the wetting line segments adopt the minimum possible inclination such that

$$\cos \quad = U/V; \qquad (V \geqslant U)$$

One consequence of this is that the minimum length of wetting line necessary to wet a unit width of the tape is

$$l = V/U; \qquad (V \geqslant U)$$

and we see that at tape speeds greater than U, the length of the wetting line must increase linearly with tape speed. The value of U obtained by fitting the data in Fig. 2 is 19.4 cm s^{-1}. At this speed, the measured contact angle is 170° rather than 180°, but the agreement with theory is reasonable in the light of experimental uncertainties. The speed at which air bubbles begin to be entrained is 25 cm s^{-1}.

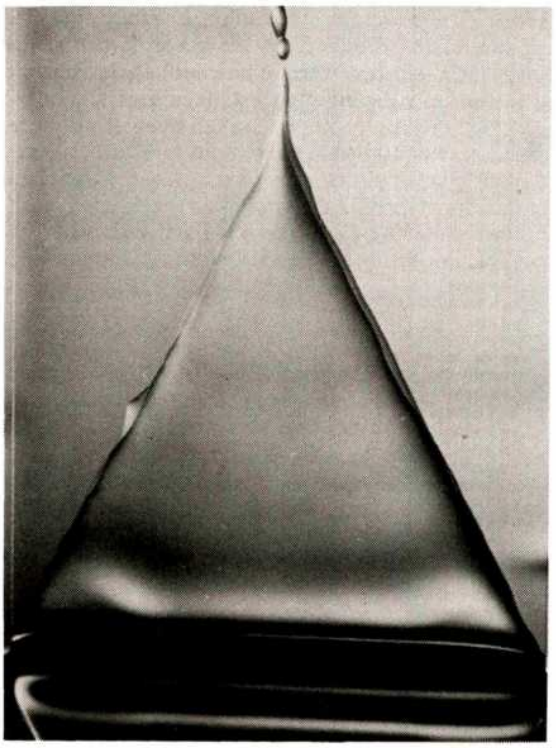


Fig. 3 Example of the sawtooth wetting line that forms when the tape is slowly withdrawn from the liquid at a steady speed greater than the maximum speed of dewetting. The tape is transparent so that two nearly identical triangular tongues of liquid can be distinguished, one on either side of the tape. Methylene blue dye was added to the liquid to increase contrast for the photograph.

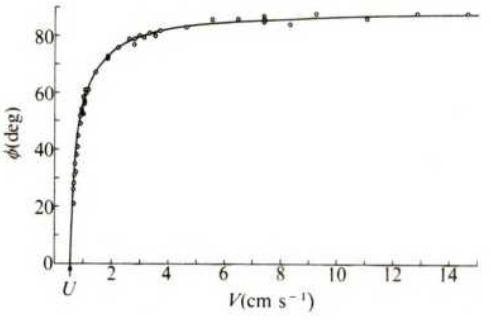


Fig. 4 Measured values of ϕ, the angle of inclination with respect to the horizontal, when the tape is withdrawn from a nominally 72% glycerol/water mixture (viscosity 0.23 P). The theoretical curve was fitted to the data to determine the maximum speed of dewetting, $U = 0.55$ cm s^{-1}.

The same phenomenon occurs when the tape is slowly withdrawn from the pool and the liquid recedes from the tape. Above a tape speed which presumably corresponds to a dynamic contact angle of 0°, the wetting line again consists of at least two straight segments slanted from the horizontal (Fig. 3), and drops or a continuous rivulet of liquid may be entrained from the trailing (higher) vertices. Measured values of the wetting line inclination for one system are shown in Fig. 4. Again, the curve is the limit of the above inequality, where U is the maximum speed of dewetting. The value of U obtained by fitting the data is 0.55 cm s^{-1}, and liquid drops begin to be entrained into the air phase at a speed of 0.8 cm s^{-1}.

The theory presented above predicts that the breakup of the wetting line will be postponed to tape speeds greater than U when the tape does not enter the liquid vertically. Specifically, the wetting line will adopt a sawtooth shape once the tape speed exceeds $U/\cos\psi$, where ψ is the angle of the tape with respect to the vertical. Preliminary experiments in which the tape entered or left the pool at a 45° angle have shown that the expected gain is in fact realised.

The theory also suggests why, with a tape plunging vertically into a pool at a speed greater than U, air bubbles form only at the

trailing vertices of the sawtooth wetting line. Presumably, the curvature of the wetting line is large but finite where two straight-line segments seem to intersect. Thus, the tangent to the wetting line at this apparent vertex is horizontal, and this point of the wetting line will be drawn into the liquid since its speed relative to the tape cannot exceed U. On the other hand, the leading vertices will tend to recede and become trailing vertices, accounting perhaps for the unsteadiness of the sawtooth wetting line when leading vertices are present.

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A CO_2-climate sensitivity study with a mathematical model of the global climate

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An increase in the CO_2-content of the atmosphere resulting from man's activity could have a significant effect on the climate in the near future[1]. We describe here some new results from a study of the response of a mathematical model of the climate to an increase in the CO_2-content of the air.

The mathematical model consists of (1) a general circulation model of the atmosphere and (2) a simple mixed layer ocean model with uniform thickness. The atmospheric model predicts the changes of the vertical component of vorticity, divergence, temperature, moisture and surface pressure based on the equations of motion, the thermodynamical equation, and the continuity equations of moisture and mass. The horizontal distributions of these variables are represented by a limited number of spherical harmonics (maximum zonal wavenumber retained is 15)[2,3]. However, the vertical distributions are specified at nine unequally spaced finite difference levels. The model has a global computational domain and realistic geography.

For the computation of solar and terrestrial radiation, the distributions of ozone and cloud cover are prescribed beforehand and the concentration of carbon dioxide is set differently for each experiment, whereas the distribution of water vapour is determined from the prognostic system of water vapour.

Condensation of water vapour is predicted whenever supersaturation is indicated in the computation of the continuity equation of water vapour[4]. Snowfall is predicted when air temperature near the Earth's surface falls below the freezing temperature[5]. Otherwise, rainfall is predicted.

The temperature of continental surface is determined so that it satisfies the requirement of the heat balance[5]. The changes of soil moisture and snow depths are obtained from the budget-computations of water and snow, respectively[5].

The ocean model is a static isothermal water layer of uniform thickness with provision for a sea ice layer. The thickness of 68 m is chosen to ensure that the heat storage associated with the annual cycle of observed sea surface temperature is correctly modelled. Ocean temperature change is computed based on the budget among surface heat fluxes. In the presence of sea ice, the temperature of underlying water is at the freezing point and the heat flux through ice is balanced by the latent heat of freezing and melting at the bottom of the ice. This process, together with the melting at the ice top, sublimation and snowfall, determines the change of ice thickness[6]. The albedo of sea ice and continental snow is assumed to vary between 60 and 70% depending on latitudes. Smaller values are assigned for thin sea ice or thin snow or melting sea ice.

To evaluate the climatic effect from an atmospheric CO_2 increase, we perform two time integrations of the model assuming the normal seasonal insolation cycle and starting from an isothermal, dry and motionless atmosphere with isothermal ocean. The atmospheric CO_2 concentration is set at 300 p.p.m. and 1,200 p.p.m. by volume, respectively (hereafter, these experiments are referred to as $1\times CO_2$ and $4\times CO_2$ experiments). Both cases settle down to a stable climatic condition in about a decade of model time (see Fig. 1). The standard experiment successfully reproduces the observed basic characteristics of geographical and seasonal temperature variation, encouraging the assertion of realism for the model sensitivity to CO_2 changes. The seasonal variations of the model climate discussed below represent mean annual cycles over the last 3-yr periods of both time integrations.

Figure 2*a* shows the seasonal variation of the difference of zonal mean surface air temperature between the $4\times CO_2$ and $1\times CO_2$ atmospheres. In low latitudes, the warming due to the quadrupling of CO_2 content in air is relatively small and depends little on season, whereas in high latitudes, it is generally larger and varies markedly with season particularly in the Northern Hemisphere. Over the Arctic Ocean and its neighbourhood, the warming is at a maximum in early winter and is small in summer. This implies that the range of seasonal variation of surface air temperature in these regions reduces significantly in response to the quadrupling of CO_2 content of air. From the seasonal variations of zonal mean sea ice thickness shown in Fig. 3, it is observed that the sea ice from the $4\times CO_2$ experiment is everywhere less than the sea ice from the $1\times CO_2$ experiment. Therefore it is reasonable to suggest that the $1\times CO_2$ atmosphere is insulated by thicker sea ice from the influence offrlying seawater and has a more continental climate with a larger seasonal variation of temperature than the $4\times CO_2$ atmosphere. Although the poleward retreat of highly reflective snowcover and sea ice is mainly responsible for the relatively large warming

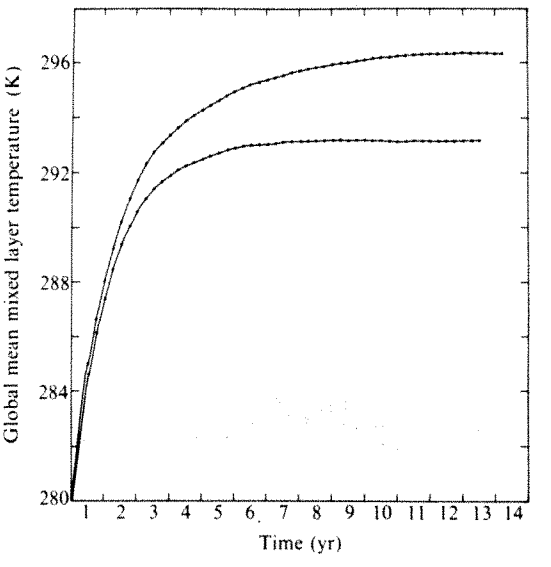


Fig. 1 Time variation of the global mean mixed layer temperature. Lower and upper lines are obtained from the $1\times CO_2$ and $4\times CO_2$ experiment, respectively. The seasonal variation is removed through the application of the running mean procedure over the period of 12 months.

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in high latitudes, the change of the thermal insulation effect of sea ice strongly influences the seasonal variation of the warming over the Arctic region.

An analysis of the heat balance over the Arctic Ocean indicates that, in early winter, the flux of sensible heat from the Earth's surface to the atmosphere is much larger in the $4\times CO_2$ case than the $1\times CO_2$ case because of the difference in the magnitude of upward conductive heat flux through sea ice. As the stable stratification in the surface layer of the model atmosphere prevents the sensible heat flux from penetrating into the middle troposphere in high latitudes, the aforementioned difference in sensible heat flux results in the large warming of surface air during early winter. As previously stated, the magnitude of the warming in the summer is much less than the warming in the winter. Because sea ice is thin or absent during summer, the surface albedo reduces significantly and net incoming solar radiation increases from the $1\times CO_2$ to the $4\times CO_2$ case. However, the additional solar radiation is used either for melting upper surface of sea ice or warming the ice-free mixed layer which has a large heat capacity. Thus, the summer warming of the surface air turns out to be relatively small. However, the additional solar energy, which is absorbed during summer in the

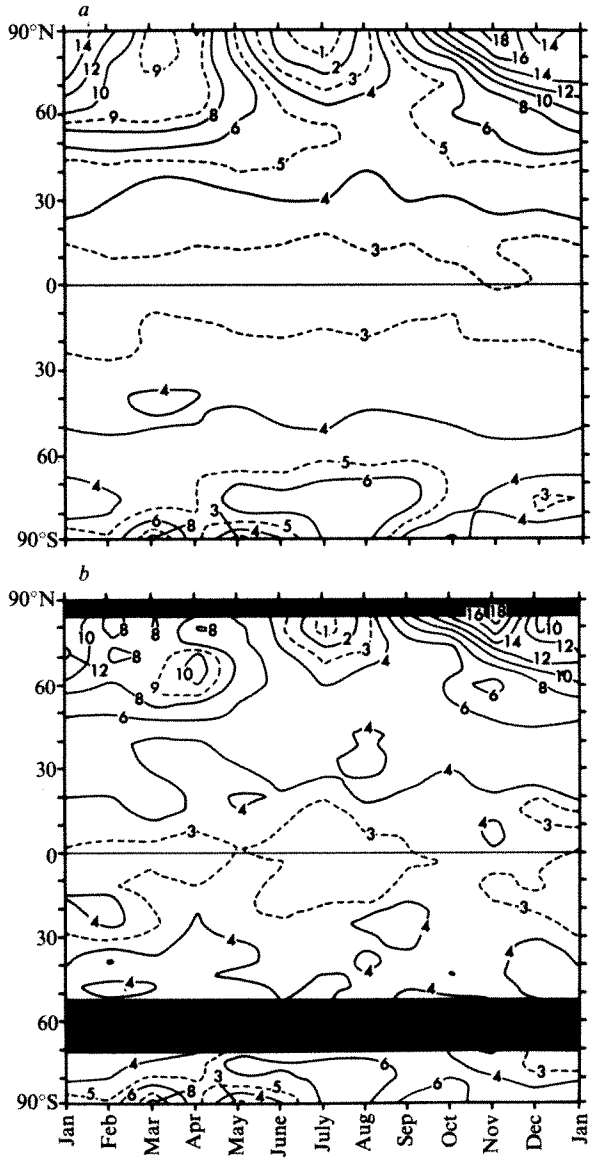


Fig. 2 Latitude–time distributions of the difference in zonal mean surface air temperature between the $4\times CO_2$ and $1\times CO_2$ atmospheres. *a*, Oceans and continents; *b*, continents only. (Positive value indicates warming caused by the quadrupling of CO_2-content of air.)

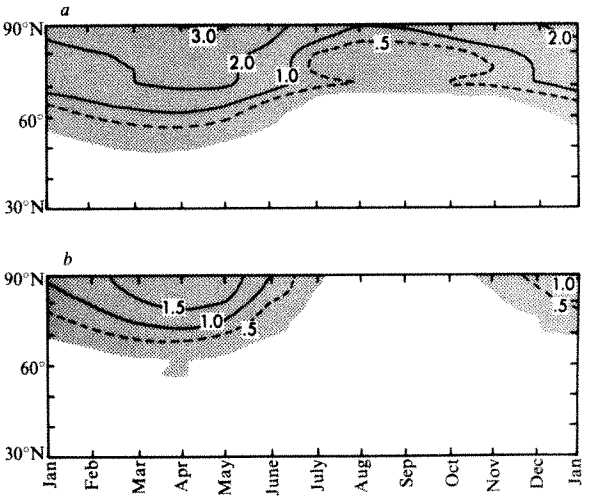


Fig. 3 Latitude–time distributions of zonal mean thickness of sea ice in metres for the Northern Hemisphere oceans. *a*, $1\times CO_2$ experiment; *b*, $4\times CO_2$ experiment. The shading indicates the areas where sea ice thickness is more than 0.1 m.

$4\times CO_2$ case, delays the appearance of sea ice or reduces its thickness. This increases the conductive heat flux through ice in early winter when the air–sea temperature difference becomes large, thereby enhancing the warming of the surface atmospheric layer in early winter.

The seasonal variation of the difference in the surface air temperature between the two experiments over the model continents is significantly different from the variation over the model oceans. According to Fig. 2*b*, which shows the latitude–time distribution of the difference in zonal mean surface air temperature over continents, the CO_2-warming in high latitudes is at a maximum in early winter, being influenced by the large warming over the Arctic Ocean discussed above. However, Fig. 2*b* also indicates a secondary centre of relatively large warming around 65°N in April. This results from a large reduction in surface albedo in spring when the insolation acquires a near-maximum intensity. The reduction of snow cover area from the $1\times CO_2$ to the $4\times CO_2$ experiment is responsible for this albedo difference.

This study shows two interacting mechanisms, each acting to produce its own sensitivity maximum. The maximum warming of the early winter over the Arctic Ocean and its neighbourhood is caused by the change in sea ice thickness, and the relatively large warming over the continents in spring is produced by snow albedo feedback. Therefore, it seems reasonable that simpler climate models with the albedo feedback mechanism but without the thermal insulation effect of sea ice would have the largest sensitivity in the early summer[7,8]. Using his simple model, Sellers[9] found an early winter maximum sensitivity at ~80°N. This winter maximum, however, apparently results from different mechanisms.

Table 1 summarises the area mean changes of the annual mean surface air temperature of the model atmosphere which occurs in response to the quadrupling of CO_2 content in air. According to Table 1, the global mean warming of the model

Table 1 The surface air temperature difference between the $4\times CO_2$ and $1\times CO_2$ atmosphere in °C

	Northern Hemisphere	Southern Hemisphere	Global domain
Difference	4.5	3.6	4.1

Positive value indicates warming caused by the quadrupling of CO_2-content of air.

atmosphere is ~4°C. This result suggests that the warming caused by the doubling of CO_2-content would be ~2°C. This is significantly less than the warming which is estimated by the general circulation model of Manabe and Wetherald[10] with idealised geography and without seasonal variation of insolation. Table 1 also reveals that the area mean warming of the Northern Hemisphere is significantly larger than that of the Southern Hemisphere. This interhemispheric difference results partly from the smallness of the snow albedo–feedback effect over the Antarctic ice sheet where surface albedo differs little between the $1 \times CO_2$ and $4 \times CO_2$-experiments.

In view of the assumption of fixed cloudiness and various simplifications contained in the sea ice modelling, the quantitative aspect of the present results should be received with caution. However, this study suggests that the warming of the atmosphere in response to an increase in CO_2-content of the air will have significant seasonal and interhemispheric asymmetries.

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Carbon disulphide and carbonyl sulphide from biogenic sources and their contributions to the global sulphur cycle

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Estimates of the magnitude of biogenic sulphur emissions range from ~70% of the total atmospheric sulphur burden[1–7], and the chemical nature of the emissions has not been clearly established. Conway[1] speculated that the principal volatile biogenic component of the sulphur cycle was hydrogen sulphide (H_2S) whereas Lovelock *et al.*[8] and Rasmussen[9] have suggested that dimethyl sulphide (DMS) contributes to the apparent source deficits. Aneja *et al.*[10] have shown that both H_2S and DMS are emitted from saltmarshes. Other volatile sulphur compounds which may contribute to the sulphur burden of the atmosphere include methyl mercaptan (CH_3SH)[11,12], dimethyl disulphide ($(CH_3)_2S_2$)[11,12], carbonyl sulphide (COS)[13] and carbon disulphide (CS_2)[14,15]. We report here the discovery of CS_2 and COS emanating from a saltmarsh and estimate their emission rates using emission flux reactor and bag chamber techniques. The species CS_2 and COS are relatively inert in the troposphere, so may be assumed to penetrate to the stratosphere, where they may be photolysed to form the sulphur dioxide (SO_2) and sulphates (SO_4^{2-}) known to be present in the stratosphere. Based on the measured fluxes, we show that the emissions from marshes are important to the sulphate aerosol burden ($\leq$19%) of the stratosphere, but not important for the tropospheric sulphur burden ($\leq$0.2%).

An emission flux reactor (chamber) was used for measuring Earth–atmosphere fluxes of biogenic sulphur compounds. The chamber technique has high sensitivity for flux measurement without the necessity of measuring very low concentrations. In addition, gas residence times in the emission flux reactor are of the order of minutes so that chemical transformations between emission and analysis may be minimised. The chamber used by Aneja[11] and Hill *et al.*[12] was modified by making the walls from 5-mil (~0.13 mm) thick FEP Teflon supported by an exterior aluminium frame. This design was chosen to provide negligible attenuation of ambient light.

The analytical instrument used was a gas chromatograph (Tracor 270HA) equipped with a flame photometric detector. A 394-nm interference filter made the detector sensitive to sulphur compounds. A 36-foot (~11 m) FEP Teflon column ($\frac{1}{8}$ inch (0.32 cm) o.d.) packed with 40–60 mesh Teflon coated with 5% polyphenyl ether (PPE) and 0.5% phosphoric acid was used at 50 °C to separate gaseous sulphur species[17]. The carrier gas was nitrogen with a flow rate of ~81 ml min^{-1}. The retention time for CS_2 in these conditions is ~14 min which allows a good separation from $H_2S + COS$ (1.5 min), SO_2 (3.1 min), and CH_3SH (5.0 min). The overlapping DMS (12.2 min) and CS_2 peaks were manually resolved into separate contributions. This column did not resolve H_2S and COS, so these species were separated by incorporating a second 19-foot (5.8 m) FEP Teflon column ($\frac{1}{8}$ inch (0.32 cm) o.d.) packed with 50/80 mesh Porapak QS in parallel with the first. The two columns were incorporated in the flow lines separately by a pneumatically controlled switching system. The carrier gas for the second column was nitrogen with a flow rate of ~40 ml min^{-1}. The retention time for COS in these conditions is ~8 min, which allows a good separation from H_2S (retention time ~5.5 min); however, this column retained CS_2. The gas chromatograph was calibrated for both columns in the laboratory and in the field using a dilution system and bag samples.

Intensive measurements were made for biogenic sulphur species at Cox's Landing, Long Beach, North Carolina; and at Cedar Island Wildlife Refuge, North Carolina, which are saltmarshes on the east coast of the US (Fig. 1). Experiments were performed during late summer at both locations. At Cox's Landing, 12 different test sites were selected. At each test site, two identical emission flux reactors were placed next to each other. One was flushed with pure gaseous nitrogen (de-aerated) and the other with ambient air (aerated). This was done to gain information on the oxygen-depleted gaseous environment, as well as to maintain the integrity of the emitted sulphur compounds in the de-aerated case. Sampling was done over mud flats, over regions close to marsh grass where the vegetation was decaying, and over marsh grass (*Spartina alterniflora*) clipped to about 1 inch above ground level. Time-integrated bag samples of the effluent gas from the emission flux reactor were collected every 30 min. These bag samples were analysed within 30 min for various gaseous sulphur species in a controlled environment mobile laboratory parked at the edge of the marsh. All the experiments at Cox's landing were performed to study the emission fluxes of CS_2 for diurnal and tidal variations. During the experiments, the ambient temperature ranged between 19.1 and 33.4 °C, sediment temperature with the tide out ranged from 24.2 to 32.6 °C, and the water temperature with the tide in was between 23.8 and 31.2 °C.

Figure 2*a* is a typical chromatogram (using the PPE column) of sulphur gases emitted from the saltmarsh. It indicates the presence and identity of CS_2 and various other biogenic sulphur species.

The average emission rates of CS_2 at the various types of test sites are given in Table 1. In 20 of the 172 aerated runs, CS_2 was detected; and CS_2 appeared only one in the de-aerated case. All of the data in Table 1 are for the aerated case. The CS_2 emission rates in the mud flat zone in the presence and absence of a water column for varying light conditions were less than 0.05 gS m^{-2} yr^{-1} (this is the emission rate based on the lowest detectable CS_2 concentration). CS_2 is also evolved over and in

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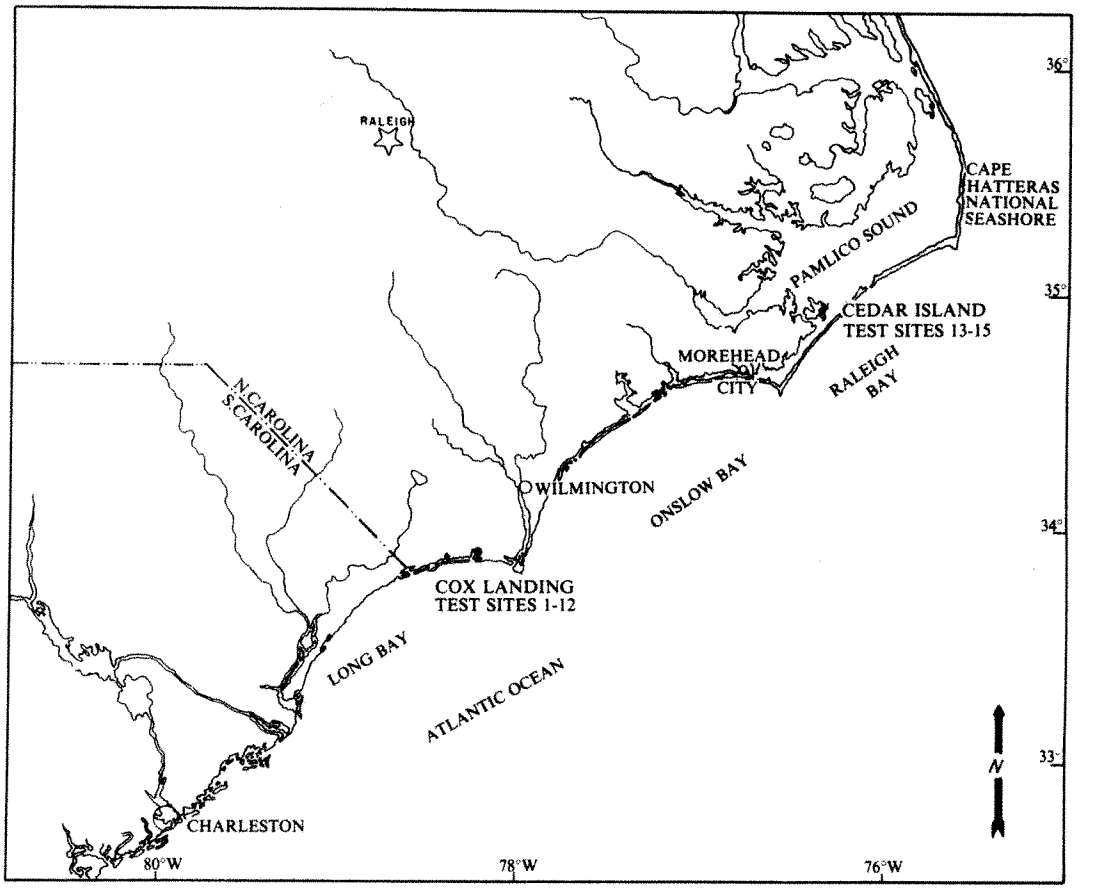
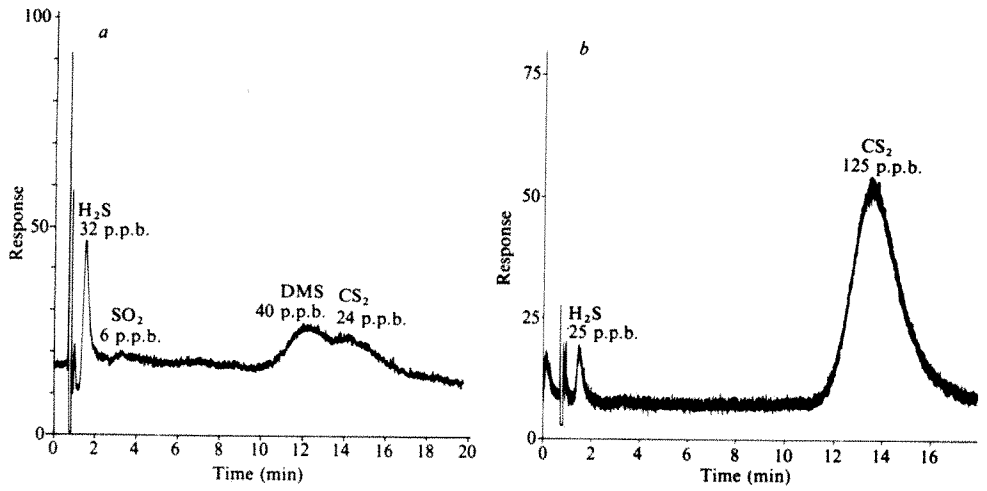


Fig. 1 *a*, Location of the sites. Sites 1–12 were at Cox's Landing and Sites 13–15 at Cedar Island.



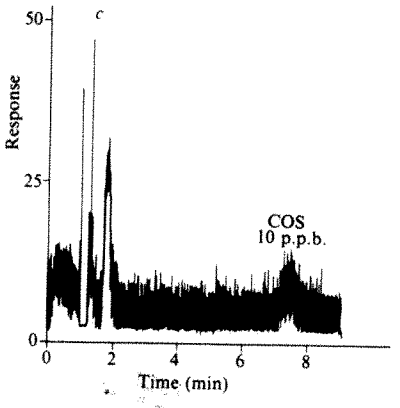


Fig. 2 *a*, Chromatogram of volatile sulphur compounds from *Spartina* zone. The chromatogram was obtained at Cox's Landing using the PPE column and a nonlinear sulphur-specific flame photometric detector (FPD). *b*, Chromatogram of volatile sulphur compounds obtained from enclosed *Spartina* stem. The chromatogram was obtained at Cox's Landing using the PPE column and a nonlinear sulphur-specific FPD. *c*, Chromatogram of volatile sulphur compounds resolved on a Porapak QS column. The sample was obtained at Cedar Island.

Table 1 Emission rate measurements of CS_2

Test site characteristic	Mean emission rate of all runs, gS m^{-2} yr^{-1} (no. of runs)	Mean of runs with emission rate greater than detectable limit*‡, gS m^{-2} yr^{-1} (no. of runs)
Over mud flat zone (direct emission rate measurement*) August 1977	<0.05 (100)	— (0)
Over decaying *Spartina* zone (direct emission rate measurement*) August 1977	~0.04 (61)	0.19±0.07 (13)
Over *Spartina alterniflora* zone (direct emission rate measurement*) August 1977	0.13 (11)	0.21±0.06 (7)
Enclosed single *Spartina alterniflora* plant (indirect emission rate measurement†) July 1978	0.20 (10)	0.20±0.09 (10)

* Direct emission rate $n = (F \cdot \Delta C/A)$, where F = steady volumetric flow rate of gas through the chamber; ΔC = concentration increase; A = area of emitting surface covered by the chamber. Based on the CS_2 detectable limit of the instrument, the corresponding emission rate is <0.05 gS m^{-2} yr^{-1}, and the CS_2 analytical error is ±3% of the estimated value.

† Indirect emission rate $n = (S \cdot \Delta C \cdot V)/t \times 8{,}760$, where S = no. of stems per m^2; ΔC = concentration increase, parts per 10^9; V = volume of chamber (1.96×10^{-3} m^3); t = time of enclosure (h). For this measurement, the CS_2 detectable limit is 0.017 gS m^{-2} yr^{-1} and the CS_2 analytical error is ±3% of the estimated value.

‡ Values are given ±s.d.

the vicinity of *Spartina alterniflora* under the aerated emission flux reactor in the presence and absence of a water column and the fluxes range from <0.05 to ~0.3 gS m^{-2} yr^{-1} (Table 1). CS_2 may be formed at a juncture of the carbon and sulphur cycles by either biogenic or chemical activity. These observations show that CS_2 is certainly produced in an aerated environment, and that it may be formed in a de-aerated environment. Lovelock[15] has observed that CS_2 can originate in anaerobic conditions on the sea floor, while, in the *Spartina alterniflora* zone, we observe it more often in aerobic conditions. The reasons for the differences between the aerated and de-aerated chamber runs are not yet known.

These observations suggest that *Spartina alterniflora* plants may be associated with the production of CS_2. This association is also supported by the results of an indicrect method of estimating gasous sulphur flux (see ref. 18 for the enclosure method). A single stem was enclosed for ~6 h in a Pyrex glass cylindrical chamber of ~1.96 litre volume (~100 cm long and ~5 cm i.d.). Samples were collected during day and night. Ten such experiments were performed and *in situ* syringe samples (one for each stem) were withdrawn from the glass chambers and analysed on the gas chromatograph. In all cases large quantities of CS_2 were observed (see Fig. 2*b* and Table 1).

Seneca *et al.*[19] have determined the stem density in North Carolina coastal saltmarshes to be ~200±25 stems m^{-2}. Based on this result, the emission rate of CS_2 from *Spartina alterniflora* stems is estimated to be ~0.2 gS m^{-2} yr^{-1} (Table 1). Note that this indirect emission rate measurement leads to emission rate values similar to those obtained by direct flux measurement over the *Spartina* zone (Table 1).

At Cedar Island three different test sites were selected over *Spartina alterniflora* and mud flat zones. At each test site the emission flux reactor was used in the aerated mode only. All the experiments described (Table 2) were performed to study the emission fluxes of COS. It establishes the presence and identity of COS as indicated by the gas chromatogram in Fig. 2*c*. The indirect method of estimating gaseous sulphur flux revealed no release of COS from *Spartina alterniflora* plants.

To estimate the per cent of biogenic contribution of CS_2 and COS to the global tropospheric sulphur cycle, we assumed that all marshes are completely made up of *Spartina alterniflora* and that they emit uniformly. We took the total marsh area[20] as 3.8×10^5 km^2, and assumed that 100×10^6 tons of gaseous sulphur are emitted per year from the biosphere by natural processes[4]. Based on our CS_2 indirect and COS direct emission rates (~0.2 gS m^{-2} yr^{-1} and 0.03 gS m^{-2} yr^{-1}, respectively) and the above assumptions, the contribution of CS_2 and COS from marshes is a small portion, <0.07% and 0.09%, respectively, of the biogenic sulphur. Obviously, more work is needed to characterise the global processes adequately.

We also estimated the contributions of CS_2 and COS emissions to the stratospheric sulphate aerosol layer. According to Crutzen's[16] estimate, a flux of ~1.8×10^{-4} gS m^{-2} yr^{-1} into the stratosphere will account for the entire stratospheric sulphate aerosol layer. Applying Junge's[21] one-dimensional eddy diffusion model to the troposphere, and using the present CS_2 and COS average emission rate for global marshes and CS_2 and COS atmospheric lifetimes[14] of 1 yr and 20 yr respectively, an upper limit to the contributions can be made by assuming zero CS_2 and COS concentrations at 15 km height (average Junge aerosol layer ~20 km). The upper limit contributions are 8% for CS_2 and 11% for COS. However, if land plants emit CS_2 at rates comparable to those measured for *Spartina alterniflora* the biogenic emissions of CS_2 could account for the entire Junge layer. Experiments are under way to test for CS_2 emissions for land vegetation.

We thank E. Corse, R. Whitkus, M. Richards, and H. Sher of NSI for assistance.

Table 2 Emission rate measurements of COS

Test site characteristic	Mean emission rate of all runs, gS m^{-2} yr^{-1} (no. of runs)	Mean of runs with emission rate greater than detectable limit‡ gS m^{-2} yr^{-1} (no. of runs)
Over mud flat zone (direct emission rate measurement*) July 1978	0.05 (2)	0.05±0.01 (2)
Over decaying *Spartina* zone (direct emission rate measurement*) July 1978	0.01 (2)	0.02 (1)
Over *Spartina alterniflora* zone (direct emission rate measurement*) July 1978	0.03 (7)	0.04±0.03 (6)
Enclosed single *Spartina alterniflora* plant (indirect emission rate measurement†) July 1978	<0.01* (10)	<0.01* (0)

* Based on the COS detectable limit of the instrument, the corresponding emission rate is <0.01 gS m^{-2} yr^{-1} and the COS analytical error is ±20% of the estimated value. See Table 1.

† See Table 1.

‡ Values given ±s.d.

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Tidal flexure of ice shelves measured by tiltmeter

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A new design of tiltmeter has been used to measure the tidal bending of the Ronne Ice Shelf, Antarctica. By this means the point at which the ice sheet leaves the land and starts floating on the sea has been defined to within 600 m. Monitoring this position in future should give an indication of the state of balance of the West Antarctic ice sheet, often considered[1] to be unstable even if not actually disintegrating. The hydrostatic level meter is light and simple and can operate unattended while recording continuously for periods of several weeks. Observations at eight different sites on Rutford Ice Stream (Fig. 1) gave tidal records on ice more than 1,700 m thick and at points up to 600 km from the nearest open sea.

Antarctic ice shelves are floating ice sheets varying in thickness from 100 m to more than 1,700 m. They rise and fall on the ocean tide, bending over a narrow region where they join the grounded ice sheet. The variation in theoretical surface elevation profiles over the duration of a tidal cycle have been derived [1,2] for several constitutive laws, but there have been no published measurements of tidal changes in profiles. The reason is partly because there are crevasse fields in many hinge zones and partly because of the lack of a suitable measurement technique. Gravimeters, strain meters and optical methods all have serious drawbacks because of their cost or their logistic and manpower requirements. The hydrostatic level meter, however, is a stable, continuously recording and relatively cheap instrument that, once placed in a shallow trench in the snow surface to reduce temperature and atmospheric effects, can operate unattended for long periods. For a short base length (5 m in our case) the instrument will measure changes in surface gradient which can be integrated along a transect to obtain a surface profile.

There are several reasons for wanting to measure tidal flexing. Actual profiles can be compared with theoretical[1,2] profiles in order to deduce the most appropriate constitutive law. There is evidence that the crystal orientation fabric of the ice can be significantly altered as it passes across the hinge zone[3]. A knowledge of strain rates is necessary for calculating the development of the fabric[4] and its effect on the flow law. Plastic deformation of ice in the bending zone dissipates tidal energy[5], but for a precise estimate both the constitutive law and the region over which bending occurs must be known. By measuring tidal constants it will be possible to study the interaction of tide not only with the main body of an ice shelf[6] but also with its grounding region. The stability of ice sheets grounded below sea level is thought to be controlled by the shielding effect of ice shelves[7]; tidal flexing may effect the dynamic behaviour in the hinge zone and may thus be an important consideration in the possible disintegration of the West Antarctic ice sheet[8]. By locating accurately the position of the grounding line any future changes can easily be monitored.

An instrument was required which would measure tilts of the ice surface as small as 10^{-6} radians with as large a dynamic range as possible. Tiltmeters currently available were considered unsuitable mainly because of the difficulty of mounting them on ice; all were intended to be installed in boreholes to give the necessary stability. In most materials and particularly in ice, such a hole takes several days to become mechanically and dimensionally stable. We therefore decided to use an instrument which could measure the height difference between two separated points on the glacier surface. The hydrostatic level meter consists of two vessels containing a liquid connected by a tube so that the liquid surface forms a gravitational potential (Fig. 2); a second tube connects the air space above the liquid at each end. Instruments of this form have been used for many purposes, for example for precision levelling on Hawaii[9] but the liquid height has been read by direct optical means and attempts to make an automatically recording instrument using floats with displacement transducers, ultra-sonic measurement of fluid level, or mercury capicitance transducers have only been a success in laboratory conditions.

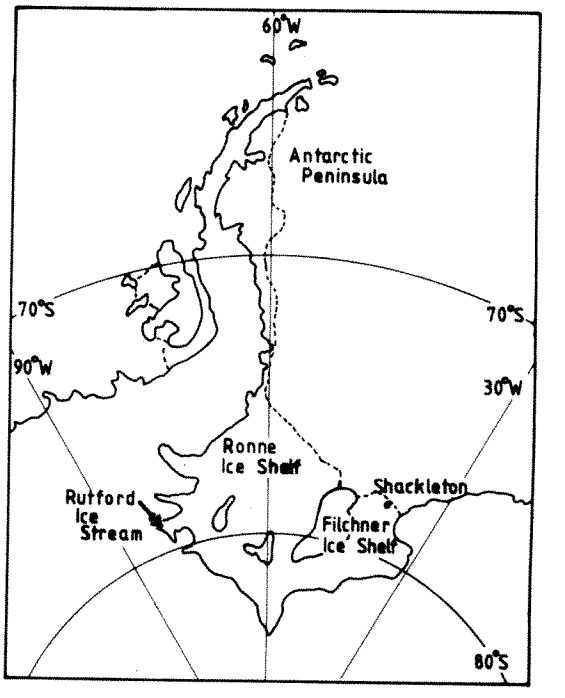


Fig. 1 Map showing location of tiltmeter sites.

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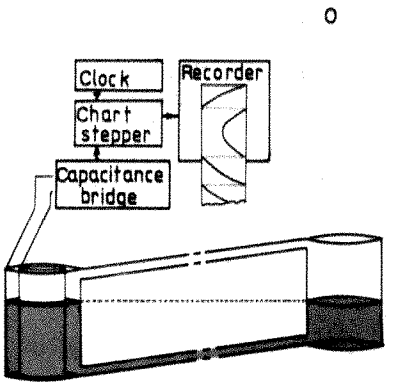


Fig. 2 Hydrostatic level meter with concentric cylinder transducer to measure liquid height.

To monitor liquid height the new instrument uses a sensitive capacitance bridge to measure the electrical capacitance between two concentric cylinders containing a liquid of known dielectric constant. Rather severe constraints on the electrical and physical behaviour of the liquid are all met by an inert fluorocarbon, ICI MFL 18. The liquid and air vent tubes are $\frac{1}{4}$-inch diameter silicone rubber tubing which does not become stiff at low temperatures. The length of the instrument was chosen to be 5 m as a compromise between the height difference signal and ease of handling; the dimensions of the measuring capacitor give a total usable range of 100 mm representing a tilt of $\pm 10^{-2}$ radians. The electronic system is the same as that of Horsfall and King's geophysical tiltmeter[10] and has a resolution of 10^{-6} in liquid height, representing tilts of 2×10^{-7} radians. It consists of a transducer drive unit, a chart stepper, a chart recorder and a quartz clock time unit; by expanding the range of the recorder by a factor of 25, the chart stepper allows full advantage to be taken of the wide range of the instrument. The complete system has a power consumption of less than 10 mA at 12 V and may therefore be run from either low temperature dry cells or, as in this experiment, from a vehicle battery.

Eight tiltmeter sites were chosen along a stake scheme which ran down the approximate centre line of the ice stream. The upper two sites showed no response to tidal bending while the third, 600 m downstream, and the rest all gave strong tidal records. The settling time until equilibrium was reached was small because the end units are light and because the instrument measures a height difference. At one site which showed no tidal movement it was possible to measure a drift which lasted for only 4 h. The shortest recording period at any of the sites which gave tidal records was 3 days. The amplitude of the vertical movement, about 10 mm, had not appreciably died away at the end site, showing that the flexure zone extended more than 6 km. The distribution of amplitudes did not show the pattern expected from considering a simple hinge line. This suggests that the glacier side walls influence the flexing, or that there are locally grounded regions within the flexing zone downstream of the first grounding line. The measured curves can be compared with a tidal prediction (Fig. 3) based on data for the four main tidal constituents (O_1, K_1, M_2, S_2) obtained at Shackleton Base on the Filchner Ice Shelf in 1957 by Pratt[11]. Although the Rutford Ice Stream is nearly 1,000 km from Shackleton Base and differs in longitude by 43°, the times of high and low water match surprisingly well.

The ease by which tidal measurements can be taken using the new tiltmeter means that the interaction between ice shelves and tides can be studied in detail. In particular, our results show that the position of the grounding line can be found with sufficient accuracy for any changes to become quickly apparent when monitored in future.

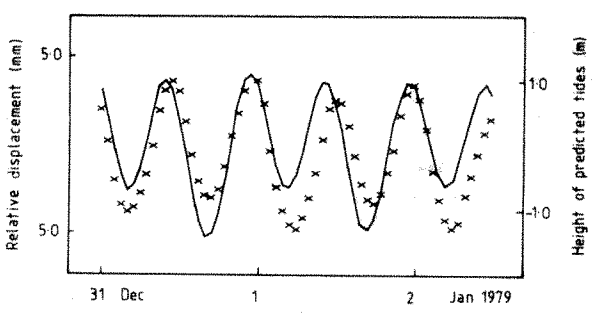


Fig. 3 Comparison between tiltmeter record (crosses) and predicted tide (solid line).

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A geologically meaningless Rb–Sr total rock isochron

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A major objective in total rock Rb–Sr isotope studies of relatively homogeneous igneous or high-grade metamorphic gneisses is to obtain as wide a range of $^{87}Rb/^{86}Sr$ ratios as possible, so that the resultant isochron may be well defined[1]. This is often achieved by collecting samples at some distance from each other. Recent studies[2–6] have shown that primary Rb–Sr isotopic systems can be profoundly disturbed, and even completely reset, in response even to low-grade secondary deformations and/or mineral alterations, and in three cases[2–4] isochron diagrams based on regionally collected samples have been shown to yield linear arrays corresponding to apparent ages intermediate between the true ages of the primary and secondary events. The evidence we present here shows that it is also possible for well defined intermediate isochron apparent ages to be produced where this sampling technique is used on rock suites which show evidence of only minor secondary alteration effects. An isochron so produced is geologically meaningless, that is, the apparent age is unrelated to any geological event.

We have recently presented the results of a detailed Rb–Sr isotope investigation of a high-grade, acid-intermediate, charnockitic orthogneiss suite at Arendal, southern Norway[4]. Eight series of samples, collected from individual outcrops, yielded apparent ages in two distinct groups, at ~1,540 Myr and ~1,060 Myr. The major conclusions were that the ~1,540 Myr age (best estimate 1,536 ± 26 Myr; $^{87}Sr/^{86}Sr$ initial ratio

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Table 1 Analytical data for regionally collected charnockite samples, Flostaøya, southern Norway

Sample	Rb	Sr	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{86}Sr$
F178	228.1	89.5	7.4775	0.84638±0.00014
F179	182.8	98.5	5.4161	0.81007±0.00006
F180	181.8	90.4	5.8822	0.81789±0.00010
F405	235.8	76.2	9.0755	0.87686±0.00010
F406	256.2	83.8	8.9944	0.87021±0.00014
F408	144.2	129.2	3.2500	0.77014±0.00012
F409	150.5	104.4	4.2302	0.78683±0.00012
F509	235.0	79.1	8.7302	0.87085±0.00014

Rb, Sr and Rb/Sr determined by X-ray fluorescence[9]. Precision of $^{87}Rb/^{86}Sr$ is ±1%. Precisions of $^{87}Sr/^{86}Sr$ quoted as 2σ standard error of mean. Details of location, petrography and analytical procedures presented previously[4].

0.70345±0.00014) reflects the formation of the high-grade charnockite mineralogy (quartz-plagioclase-orthopyroxene± K-feldspar, hornblende, clinopyroxene, biotite, garnet) and that there was subsequent disturbance of the total rock isotopic systems during a low-grade event which occurred at the same time as the intrusion of undeformed granite sheets and pegmatite dikes at 1,063±20 Myr. The only petrographically visible effects of this superimposed low-grade event are (1) partial, and often variable, alteration of orthopyroxenes to chlorite and/or serpentine, (2) minor corrosion of pre-existing biotite, and (3) incipient turbidity within some plagioclases. These mineral alterations were initiated along narrow (≤1 mm) irregularly spaced cracks which facilitated the open system behaviour. The superimposed event caused resetting to the younger age not only of all primary mineral systems investigated (plagioclase, K-feldspar, biotite, in four localities), but also of the 1–2 kg rock systems at those localities (three) where the mineral alterations are most pronounced. Both the secondary total rock ages, and all mineral ages, fall within error of the 1,063±20 Myr age obtained for one of the intrusive granite sheets. For each of the localities which suffered resetting of the total rock systems, all samples with $^{87}Rb/^{86}Sr \geq 0.8$ were displaced below the original 1,536 Myr isochron. This showed that the resetting process did not involve a simple rehomogenisation of the Rb–Sr systems within individual outcrops, but that there was a systematic change in $^{87}Rb/^{86}Sr$ and/or $^{87}Sr/^{86}Sr$.

The results presented here (Table 1) refer to a group of regionally-collected samples from the same charnockite body. They have been taken from an area of 1 km^2 at the northern end of the island of Flostaøya[4]; the area centres on one of the previously described localities (no. 4) which has suffered isotopic resetting during the secondary event. Both series of data are plotted in Fig. 1, together with the mineral data for sample 4.4. The data for the samples from locality 4 (a 10-m road cut) were presented in the earlier study[4].

The five total rock samples from locality 4 give a good fit isochron age of 1,075±122 Myr with a value for the mean square of the weighted deviates (MSWD)[7] of 1.10; the mineral (plagioclase, K-feldspar, biotite) + host rock data for sample 4.4 yield 1,036±20 Myr (MSWD = 2.69). When the mineral data are regressed together with those for all of the rocks from this locality, an eight-point isochron age of 1,035±9 Myr (MSWD = 1.19) is obtained (Fig. 1). These ages are all within error (2σ) of the 1,063±20 Myr age for the intrusive granite sheet, and they correlate with the low-grade superimposed event. The regionally collected samples define a good-fit apparent age of 1,264± 25 Myr. An MSWD of 1.58 indicates that the scatter about the isochron can be assigned to experimental error. The problem is that this good-fit 1,264 Myr isochron apparent age correlates with neither the primary (~1,540 Myr) nor the secondary (~1,060 Myr) event, and there is no geological evidence for an intermediate event to which it might relate. We interpret the 1,264±25 Myr isochron 'age' as being geologically meaningless.

A key aspect of these data is that a combination of the locality 4 samples with the regional samples yields an apparent age of 1,259±24 Myr (Fig. 1), which is virtually identical to that given by the regionally collected samples alone; the MSWD value, although slightly higher at 2.07, is still indicative of reasonable fit. The implication is obvious: in a regionally-based sampling programme, any individual sample collected from locality 4 would plot on, or very close to, the geologically meaningless isochron, despite the fact that the within-locality age is quite different. This situation arises because of the relatively small dispersion of $^{87}Rb/^{86}Sr$ at locality 4.

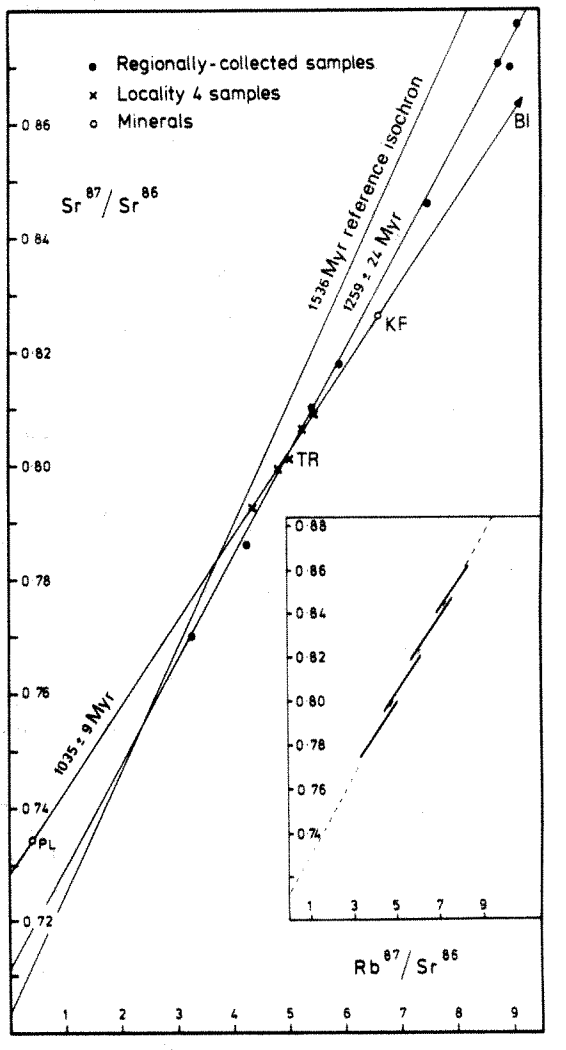


Fig. 1 Rb–Sr isochron diagram for regionally collected rock samples (●), and minerals (○) and rocks (×) from a single locality (no. 4). The 1,536±26 Myr reference isochron (IR = 0.70345± 0.00014) relates to the formation of the high grade charnockite mineralogy[4]. The 1,035±9 Myr isochron age (MSWD = 1.19; IR = 0.72862±0.00024) is that given by a combination of rocks and minerals at locality 4. The 1,259±0.00024 Myr apparent age (MSWD = 2.07; IR = 0.71189±0.00182) is that given by a combination of the regionally collected ($n = 8$) and locality 4 ($n = 5$) rock samples. By themselves the regionally collected samples define an apparent age of 1,264±25 Myr (MSWD = 1.58; IR = 0.71107±0.00194). The data have been regressed by the method of York[10]. The decay constant used is 1.42×10^{-11} yr^{-1}. All errors are quoted as $\pm 2\sigma$. The inset represents the probable relationship between the geologically meaningless 1,264 Myr isochron (dashed line) and within-locality sub-isochrons (~1,060 Myr, solid lines). The latter would reflect the true age of the superimposed event.

All of the data points for the regionally-collected samples plot below the 1,536 Myr primary reference isochron (Fig. 1). Because (1) the displacement is in the same sense as that shown by samples with $^{87}Rb/^{86}Sr > 0.8$ from all of the localities (including locality 4) which are known to have equilibrated to the 1,060 Myr age, and (2) only the two apparent age groups (~1,540 Myr and ~1,060 Myr) were revealed by the within-outcrop sampling[4], we interpret the present positions of the regionally-collected samples as meaning that each was collected from a subvolume which suffered disturbance or resetting during the ~1,060 Myr superimposed event. If each of these isotopic systems was completely rehomogenised, the sub-volumes would actually describe a number of parallel sub-isochrons corresponding to the ~1,060 Myr event, as occurs at locality 4. Moreover, if it is assumed that each sub-volume would most likely be characterised by a limited range in $^{87}Rb/^{86}Sr$ such as occurs at locality 4 and elsewhere[4], any sample taken from any locality would plot approximately along the 1,264 Myr, but geologically meaningless, apparent isochron. The model is schematically illustrated in the inset to Fig. 1. From this, it is evident that certain combinations of regionally collected samples would probably give rise to significant scatter, in which case it could at least be deduced that there had been some isotopic disturbance. More importantly, the data presented here show that regional sampling in this type of geological situation can also produce a combination which yields a good-fit intermediate apparent isochron (here, 1,264 ± 25 Myr). Despite a lack of significant scatter this isochron does not record a real isotopic event and is, therefore, geologically meaningless.

The model we have suggested is similar to a strontium isotopic equilibration model suggested by Roddick and Compston[8], with the important difference that, in the present case, there must have been systematic changes in $^{87}Rb/^{86}Sr$ and/or $^{87}Sr/^{86}Sr$ at each locality in order to displace all of the samples below the original 1,536 Myr isochron. The sub-volumes cannot have remained closed with respect to the Rb–Sr isotopes during the 1,060 Myr event. We have argued elsewhere that the main factor was net loss of ^{87}Sr, rather than increases in the Rb/Sr ratios[4].

In the example we have described, the $^{87}Sr/^{86}Sr$ initial ratio of the meaningless isochron (0.71107 ± 0.00194) is significantly higher than that given by the rocks which define the primary event (0.70345 ± 0.00014)[4], but other evidence suggests that this is not always so and that significantly lower $^{87}Sr/^{86}Sr$ initial ratios may also be generated (data not shown).

So far, we have related the problem of geologically meaningless total rock isochrons only to regionally collected samples, but the same situation might well arise within single outcrops if there is locally a wide dispersion in the $^{87}Rb/^{86}Sr$ ratios and isotopic resetting was only achieved within smaller sub-volumes. In such cases, the only sure way to test the scale of homogenisation during resetting would be to undertake isotopic analysis of minerals and of several total rock samples from each of a number of sub-volumes within the outcrop.

Our final conclusion is the same as that recently deduced by Bell and Blenkinsop[2]: selection of samples for isochron-age analysis should not be made solely on the basis of seeking the maximum possible spread in the $^{87}Rb/^{86}Sr$ ratios.

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Visual lateralisation effect in reading Chinese characters

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Visual half-field experiments have been used to investigate perceptual differences in reading different types of writing scripts. It has been observed that tachistoscopic recognition of phonetic-based scripts (such as the English alphabet and Japanese Kana) tends to show a right visual field–left hemisphere (RVF-LH) superiority effect[1,2] whereas recognition of logographic symbols (for example, Chinese characters) tends to show a left visual field–right hemisphere (LVF-RH) superiority effect[3,4]. A cerebral orthography-specific localisation hypothesis has been proposed to account for these data. We have conducted three experiments to examine the visual lateralisation effect in reading logographic symbols such as Chinese characters. Chinese subjects in the first experiment were exposed to brief tachistoscopic presentation of a single character, and their task was to name the character as soon as possible. A LVF-RH superiority was found. In the second experiment, the stimuli were two vertically arranged characters and the subjects were asked to name the stimulus terms as soon as possible. The third experiment was the same as the second except that the task was to decide whether these character-strings were correct semantic terms with a manual response. A RVF-LH superiority effect was found in both the second and third experiments. These differential visual lateralisation results were interpreted as reflecting the function-specific property of the two hemispheres and cast doubt on the orthography-specific localisation hypothesis proposed by earlier investigators.

There has been much confusion in the literature as to whether asymmetries of function in the two sides of the brain arise from stimulus properties or from the nature of task requirements. Results of some recent clinical and experimental studies on the recognition of Japanese words have generally been used as evidence to support the former view. Among the many writing systems in the world today, Japanese is unique in that three types of script are used simultaneously to represent text. Most words in Japanese are written in Kanji, a logographic script, while grammatical particles are usually written in hiragana, and loan words (that is foreign words such as television) in katakana. The latter two are called Kana scripts and each symbol represents a syllable of the spoken language. So, a fluent reader of Japanese has to know all three types of script. Sasanuma and her associates[5] have presented evidence that the ability of Japanese aphasic patients to use the Kanji and Kana scripts may be selectively related to type of aphasic disorder. Moreover, such differential properties can also be demonstrated in visual half-field experiments on normal subjects, with a LVF-RH superiority for the recognition of Kanji characters and a RVF-LH superiority for the Kana symbols. The latter result is similar to those obtained with alphabetic writings. This implies that the underlying reason for this difference is the requirement of visual-to-sound mapping. Although there is indeed evidence for the left hemisphere specialisation for speech sound[6], generalisation of such findings to explain the differences between reading logographic symbols and reading alphabetic/syllabary symbols may be misleading. There is much evidence that reading logographic symbols also requires speech recoding[7]. Thus, what the hemispheric difference found in the tachistoscopic recognition of Kanji and Kana (or alphabetic) symbols reflects is not an

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Table 1 Mean numbers of correct identification as a function of type of characters and of visual field

	Pictograms	Phonograms
Right visual field	5.20	5.60
Left visual field	13.30	14.20

The term 'lateralisation' refers to the specialisation of the left or right hemisphere of the brain for different functions. The rationale behind the visual hemi-field experiment is as follows. When a subject looks at a fixation point in the centre of a lighted square within a tachistoscope, each visual hemi-field projects to the contralateral hemisphere. So, for example, stimuli presented to the RVF are first processed in the left hemisphere. If language is indeed processed in the left hemisphere, then verbal stimuli presented to the RVF should take less time to respond than when the same materials are presented to the LVF. The delay in reaction time is attributed to the time it takes to transfer information from one hemisphere to the other. Or the experimenter can shorten the exposure duration so that the subjects are expected to make identification errors. Depending on the pattern of such an accuracy measure (that is RVF or LVF superiority) and on the materials used, specific functions of the left or right hemispheres can be inferred.

orthography-specific property; rather, it reflects a task-specific property of our cerebral hemispheric processing.

In our first experiment, 80 stimulus characters were selected from established norms of commonly used Chinese characters (both abstract and concrete terms were used). Two types of characters were selected: phonograms, which contain a phonetic component as a clue to the sound of the characters, and pictograms, which are pictographic in origin[8]. Figure 1 presents some examples of these two types of character. The subjects were 20 Chinese students (all right-handed) enrolled in the graduate school of the University of California, Riverside. The stimulus characters were presented one at a time either in the RVF or on the LVF by means of a three-channel tachistoscope. Each character was always preceded by an auditory signal plus a 500-ms fixation point presented in the centre of the screen. The duration of character presentation was adjusted for each subject according to his/her recognition threshold determined before the experimental session (range 20–75 ms; mean 40 ms). The subject was asked to identify verbally the stimulus character as soon as it was presented. The entire experiment forms a 2 (phonogram compared with pictogram) × 2 (RVF compared with LVF) design with both factors as repeated measures, and the dependent measure was the number of correct identifications. The results are summarised in Table 1. A 2 × 2 analysis of variance (ANOVA) was performed on the data. Only one main effect of the visual field is statistically significant, $F(1, 19) = 49.56$, $P < 0.001$. Apparently a strong LVF-RH superiority effect was obtained regardless of whether the characters contained any phonetic cue.

Although the result of a RH processing is clearcut, its implication for reading is less clear. Modern Chinese tends to be multiple-syllable, and so the perceptual unit in reading may be much larger than the single characters. Thus, a major task in reading is to generate meaning by putting together several characters to form meaningful terms. The second experiment, therefore, was conducted to investigate whether the results obtained in the previous experiment would be generalised to the tachistoscopic recognition of multiple-character terms. The procedure was the same as for the first experiment except that the stimulus terms were always two characters arranged vertically in either LVF or RVF of the tachistoscope. The results showed that of the 80 stimulus terms presented (40 for each field) the numbers of correct identification were 36.5 and 22.5 for stimulus terms presented in the RVF and LVF, respectively. The difference is statistically significant, $t(19) = 7.49$, $P < 0.001$. This result suggested a LH dominance, a complete reversal of the previous experiment. Therefore, the locus of the cerebral lateralisation cannot be on the logographic symbols *per se*; rather, it must be on the type of task a subject has to perform to meet the experimental requirement.

One may still object to the result of the second experiment on the basis that a LVF-RH effect as observed in the first experiment might be overridden by the LH function (it is conceivable that more effort is required to pronounce two characters) of motor programming in the oral report. To circumvent this objection, a third experiment was run to investigate the visual lateralisation effect with the subject making a semantic decision on multiple-character terms. Twenty Chinese graduate students were asked to make a 'yes/no' response as quickly and as accurately as possible to stimulus terms briefly exposed in either the RVF or LVF of the tachistoscope. They were asked to press the 'yes' key only if the characters formed a meaningful term, otherwise to press the 'no' key. The right-hand or left-hand responding to the 'yes' or 'no' key was counterbalanced across subjects. We also manipulated the imagery value by dividing the stimulus terms into 16 high and 16 low imagery terms. Thirty-two meaningless character strings were constructed to balance the positive and negative instances. The dependent measure was the reaction time (RT) required to make a correct decision. The results are summarised in Table 2.

First, subjects took much longer to make a negative response than a positive response and the data on negative responses were much noisier than those on the positive responses. Because our interest is in the positive reponse in various experimental conditions, ANOVA for a 2 (RVF compared with LVF) × 2 (high compared with low imagery) factorial design with both factors as repeated measures was performed on RTs of the correct positive responses. The main effect of imagery is significant with the high imagery terms having a 62-ms advantage over the low imagery terms, $F(1, 19) = 5.69$, $P < 0.05$. This is consistent with previous findings[9]. The main effect of visual field is also highly significant, $F(1, 19) = 14.52$, $P < 0.002$, with RVF performance having a 104.1-ms advantage over the LVF performance. In other words,

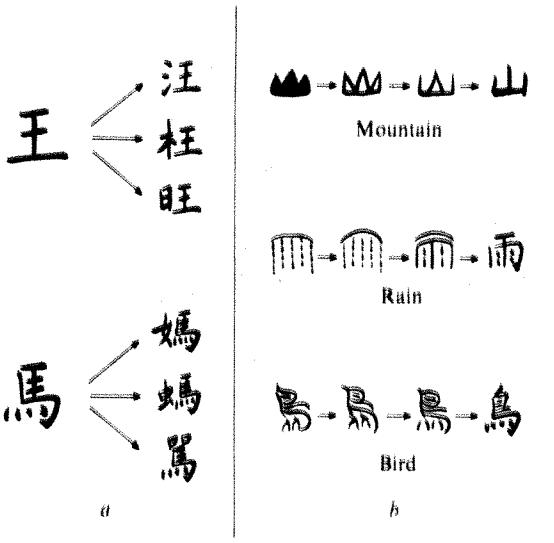


Fig. 1 *a*, Examples of Chinese phonograms. In the upper panel, the character on the left hand side is the base character and is pronounced as 'wáng' (meaning 'king'). The three characters on the right are derivatives which contain the base character as a clue to their pronunciation. In fact, they are pronounced as 'wāng', 'wǎng' and 'wàng' from top to bottom, meaning 'the barking sound of dogs' (or alternatively, 'deep and wide'), 'not straight', and 'prosperity' for the three characters, respectively. In the lower panel, the base character on the left is pronounced as 'mǎ'. It means 'horse' and it is a pictogram by itself[8]. Similarly, the three derivative characters on the right are pronounced as 'mā', 'má' and 'mà', meaning 'mother', 'ant' and 'to scold', respectively. Thus, if a reader knows how to pronounce the base characters, he can guess the pronunciation of the derived phonograms which contain the base character as its partial component. However, one should be cautious in making generalisations because in a lot of cases the base character only gives a clue to how a particular phonogram sounds (sometimes the clue refers only to the vowel ending) and the tonal patterns (—, ⁄, ∨, ∖) are not included. *b*, Examples of the pictograms and their transformation through hundreds of years.

Table 2 Mean reaction times for correct decision as a function of imagery value and of visual field

	Positive response		
	High imagery	Low imagery	Negative response
Right visual field	827.82	863.18	1,382.95
Left visual field	906.34	993.52	1,391.32

Responses are given in ms.

the results showed a RVF-LH superiority effect, replicating the result found in the second experiment.

Superficially, this result of a LH dominance seems to be in conflict with those of the first experiment. However, these data are compatible with the current view of cerebral organisation which maintains that, for most right-handed people, the left hemisphere is better at handling sequential and analytical tasks while the right hemisphere is better at handling holistic pattern recognition tasks[10]. Because the experimental task in the first experiment emphasises a holistic recognition of the single characters and because the experimental task in the second and third experiments emphasises putting character strings together to get at the lexemes of the terms, the results of these three experiments not only do not conflict with one another, but rather they support the differential-function views of cerebral organisation. Table 2 shows that the data on the imagery variable are consistent with this interpretation.

According to this view, the right hemisphere is superior in perceiving overall form. Clearly, the LVF-RH advantage for single-character recognition must have derived from this source, although final pronunciation of the meaning of the character must be mediated by the left hemisphere. This implies that the left hemisphere's articulatory superiority is outweighed by the right hemisphere's form-perception superiority for the single-character test. It seems evident that the reading and interpretation of Chinese characters calls on the specialised functions of both sides of the brain, and that whether one or the other side manifests superiority in a particular task depends on the balance of cognitive requirement.

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Spider feeding behaviour optimises dietary essential amino acid composition

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Classical models of foraging behaviour[1–3] assume that feeding patterns maximising the net caloric gain per unit time or minimising the time spent in foraging will be favoured by natural selection. There is some evidence for this behaviour in some foragers[4], mostly higher vertebrates for which time and energy constraints are frequently paramount. However, while these models may suffice under some circumstances, nutritional information from the veterinary, wildlife management, and entomological literature shows that an animal requires a diet containing all necessary nutrients to be truly fit (physiologically and evolutionarily). Therefore unless animals are very severely constrained energetically, foraging behaviour should lead to nutritional as well as caloric optimisation. Here I show that free-living wolf spiders will tend to prey on three species in proportions which optimise the proportions of the essential amino acids they provide in the diet.

The small (<50 mg) lycosid, *Pardosa ramulosa* (McCook), is associated in California with standing water, where it feeds predominantly on aquatic insects[5]. In the Petaluma Marsh in Sonoma County the spiders are found at isolated brackish pools. Eighty per cent of their diet comprises three insect species, the shore fly, *Ephydra riparia*, the waterboatman, *Trichocorixa reticulata*, and the mosquito, *Aedes dorsalis*[5].

Information on short-term dietary selectivity in the presence of a particular set of prey availabilities was obtained by statistical analysis of qualitative data on spider stomach contents, which were identified with a passive haemagglutination inhibition assay[6]. Because the assay has perfect specificity, each spider may be unambiguously scored either positive or negative for the presence of each of the three insect species. On this basis, each spider in a sample may be assigned to one of the eight cells of a $2 \times 2 \times 2$ contingency table for a test of independence among the prey species in the diet, using the marginal totals to compute the expected numbers of spiders in each cell. The null hypothesis

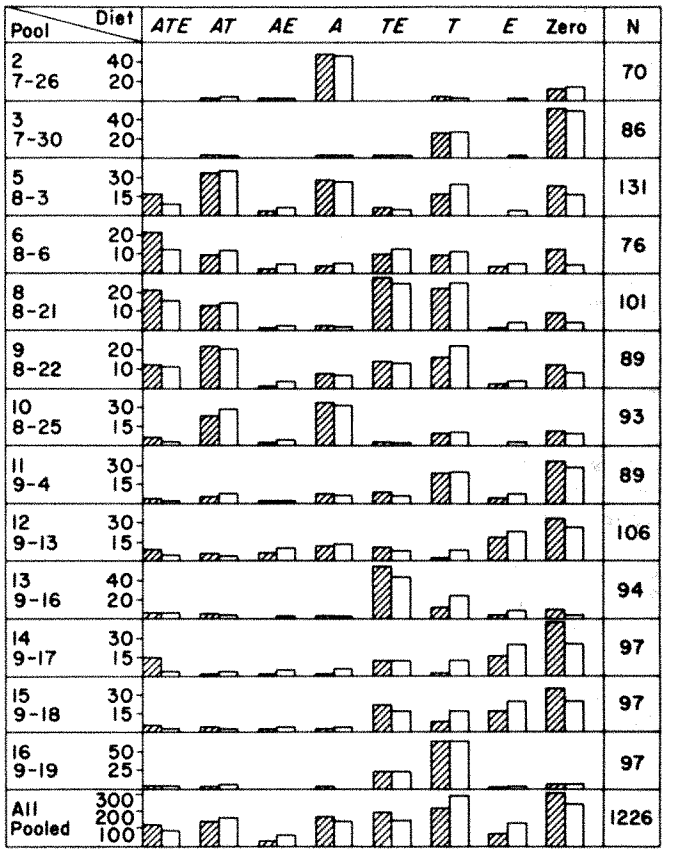


Fig. 1 Observed (hatched bars) and expected (open bars) numbers of spiders with each of the eight possible kinds of diets. Symbols are as in Table 1. A certain proportion of the spiders in the contingency table (the 'zero' category) are expected to be negative for all three species. In addition to these animals, a subset of the sample, whose identities are unknown, will not be feeding because they are preparing to moult[13]. Three per cent of the spiders in a laboratory population were not eating at any given time because of preparation for moult (unpublished data). Therefore, 3% of each sample, as zeroes, were omitted from the analysis in order to avoid an artificial inflation of the observed number of zeroes in the contingency table.

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Table 1 *G* values and their associated probabilities for the contingency table tests of independence of the prey species in the diet

Pool	T×A Independence	T×E Independence	E×A Independence	A×T×E Interaction	A×T×E Independence	*N*
2	1.499	0.118	0.523	0.000	2.141	70
3	0.139	1.997	0.048	0.000	2.184	86
5	1.367	10.906§	1.912	4.463†	18.647§	131
6	6.802†	6.802‡	3.375	0.032	17.012§	76
8	1.107	10.684§	0.892	0.371	13.055§	101
9	1.553	4.637†	0.451	0.045	6.686	89
10	0.031	3.594	0.305	1.352	5.283	93
11	0.009	3.974†	1.408	0.007	5.399	89
12	4.070†	8.412§	0.006	1.312	13.799§	106
13	0.370	19.111§	0.697	0.150	20.327§	94
14	24.821§	27.143§	18.481§	0.001	70.445§	97
15	3.607	12.946§	0.537	0.767	17.857§	97
16	1.394	0.163	0.163	0.045	0.885	97
All samples pooled:	1.461	119.992§	0.123	1.105	122.574§	1226

A = *Aedes*, T = *Trichocorixa*, and E = *Ephydra*; *N* = sample size.
† $P<0.05$, ‡ $P<0.01$, § $P<0.001$.

can be rejected in either of two ways. Positive association is consistent with a hypothesis of nutrient optimisation in which certain proportions of several species provide more nearly optimal proportions of nutrients than does any single species, or in which taking a variety of species minimises the consumption of specific noxious defensive compounds from each one[7]. Negative association of prey species in the diet would suggest specialisation on a particular species, because it provides the greatest net caloric gain or a more nearly optimal nutrient balance than any combination of species. It must be emphasised that the availabilities and dispersions of prey species on any given date and any prey species differences in catchability, visibility, and encounter rate are completely irrelevant to this analysis since they are subsumed in the marginal totals of the contingency tables.

Thirteen samples of about a hundred spiders each were collected during the summer of 1974, and the observed and expected values for the contingency tables derived from the immunological analysis of their stomach contents were compared by means of the *G* test[8] (Table 1). The pooled data, and nine of the thirteen individual samples, show significant departure from independence; in the majority of samples this is due entirely to *Trichocorixa* × *Ephydra* heterogeneity. The observed and expected values for the eight cells of each table are shown in Figure 1. All of the significant pools show an excess of triple positives and, with the single exception of pool 6, an excess of spiders positive for *Trichocorixa* and *Ephydra* and negative for *Aedes*, and deficiences of *Trichococrixa* and *Ephydra* singles. These data indicate dietary mixing.

Because of the importance of essential amino acids in the diets of arthropods[9,10], I decided to test the hypothesis that the significant positive associations of prey species reflect attempts by the spiders to provide the most nearly optimal proportions of essential amino acids in the diet. The null hypothesis is that the proportions of amino acids in the diets of the average spiders in the four nonsignificant samples (pools 2, 3, 10 and 16) are not significantly more or less similar to the optimal proportions than are the proportions of amino acids in the diets of average spiders in the nine significant samples. The test hypothesis is that the proportions of essential amino acids in the diets of the average spiders of the nine significant samples are significantly more similar to the optimal proportions than are the proportions of essential amino acids in the diets of the average spiders of the four non-significant samples.

Table 2 Proportions of the essential amino acids in the spiders and their prey

Amino acid	*Pardosa*	*Aedes*	*Trichocorixa*	*Ephydra*
Arg	0.0280	0.0437	0.0514	0.0880
His	0.0093	0.0299	0.0363	0.0281
Ile	0.1701	0.1764	0.1441	0.2168
Leu	0.2295	0.2556	0.1425	0.1735
Lys	0.0560	0.0507	0.1542	0.0545
Met	0.0147	0.0151	0.0346	0.0352
Phe	0.1054	0.2046	0.0832	0.1022
Thr	0.0869	0.0398	0.0793	0.0683
Val	0.1889	0.1827	0.1492	0.1590
Trp	0.1113	0.0014	0.1251	0.0744

Spider extracts were prepared by grinding whole animals in distilled water. Haemolymph alone was used for the insect comparisons (see ref. 6 for method of extraction) because the spiders do not consume their prey intact and therefore do not ingest skeletal material.

The optimal proportions of essential amino acids are assumed to be those in the spiders themselves. (This is obviously a first approximation since rates of utilisation may not be the same for all amino acids.) The proportion of the *i*th amino acid in the diet of the average spider in a sample is given by

$$p_i = \frac{\Sigma_j \lambda_j \mu_{ij}}{\Sigma_i \Sigma_j \lambda_j \mu_{ij}} \quad (1)$$

where λ_j is the mean number of individuals of prey species j per spider stomach in the sample and μ_{ij} is the weight of amino acid i in an individual of prey species j. The proportions of the 10 essential amino acids in the spiders and their prey (Table 2) were determined by dansylation and chromatography[11] of appropriate extracts. The λ_j values were estimated from the immunological data by assuming that the number of individuals of each prey species per spider stomach is a Poisson variate[12]. Then the probability that a stomach contains r items of species j is given by

$$p_{rj} = \frac{\lambda_j^r e^{-\lambda j}}{r!}$$

from which it follows that

$$\lambda_j = -\ln (p_{0j})$$

where p_{0j} is the proportion of spiders in the sample which is negative for species j, after correction for animals not feeding because of preparation for moult[13]. The resulting λ_j values were corrected for differences in the length of time during which individuals of the three species remain detectable in the stomach[5,14]. These corrected λ_j values (Table 3) were entered into equation (1) to compute the proportion of each of the ten essential amino acids in the diet of the average spider in each sample.

The similarity between each of the 13 average diets and the optimum diet was indexed using vector dot products. Since the proportions in any diet or in the optimum sum to 1.0, the sets of proportions can be represented as probability vectors. The dot

product of two vectors, α and β, is[15]

$$\alpha \cdot \beta = |\alpha||\beta| \cos \theta$$

In the case of probability vectors,

$$\theta = \text{arc cos} \frac{(\Sigma p_{di} p_{oi})}{(\Sigma p_{di}^2 \Sigma p_{oi}^2)^{1/2}} \quad (2)$$

where p_{di} is the proportion of the ith amino acid in the diet which is being compared to the optimum and p_{oi} is the proportion of the ith amino acid in the optimum diet. When two vectors are identical $\theta = 0°$. The more dissimilar they are the larger θ becomes, up to a maximum of 90° (ref. 16).

The θ values computed for each pool's average diet versus the optimum using equation (2) are presented in Table 3. There is no overlap between the θ values of the two sets of comparisons, all of the non-significant pools' diets' comparisons showing θ values between 19.24° and 21.92°, and all of the significant pools' diets' comparisons showing θ values between 13.11° and 18.18°, indicating diets with a closer similarity to the optimum. This difference is highly significant by the Mann–Whitney U-test[17], ($U = 0$, $n_1 = 4$, $n_2 = 9$, $P < 0.001$), and strongly supports the nutritional hypothesis.

Dietary mixing has been reported in rats[18], deer mice[19], and flour beetles[20] in the laboratory, and in songbirds in the field[21], but has not yet been described in free-living invertebrate predators. The fact that a bug (*Trichocorixa*) and a fly (*Ephydra*) were most often associated in the spiders' diet agrees with field observations that the insects most heavily represented in the diets of European[22,23] and North American[5,24] *Pardosa* species belong to the orders Homoptera, Hemiptera and Diptera. Perhaps these spiders have adopted this particular form of polyphagy for nutritional reasons. Such behaviour probably has selective value, since, in the laboratory at least, survival[25,26] and fecundity[26] are higher in *Pardosa* species raised on multiple species than on single species diets.

Recognition of the importance of nutrients besides calories has appeared in the optimal foraging literature in the past few years[27–32]. It has been suggested, for example, that protein nitrogen[27,28] or other nutrients[29] be substituted for calories as the currency for optimisation. In reality the decisions made by foraging animals will probably be better represented by more complex models entailing compromises in the simultaneous optimisation of caloric and other nutritional factors and, as shown in the present study, concerned with both the quality and quantity of nutrients.

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Table 3 λ_i Values (mean numbers of individuals of each species eaten per spider per unit time) and θ values from vector dot products, for all samples

Pool	λ_A	λ_T	λ_E	θ
2	1.50	0.12	0.01	21.97°
3	0.02	1.02	0.01	18.57°
5	1.02	1.56	0.23	18.18°
6	0.72	2.04	0.72	15.49°
8	0.51	3.50	0.67	15.41°
9	0.68	2.46	0.39	15.22°
10	1.41	1.18	0.12	19.84°
11	0.25	1.26	0.23	14.18°
12	0.42	0.62	0.60	16.54°
13	0.13	3.48	1.02	14.62°
14	0.23	0.84	0.62	14.12°
15	0.14	1.02	0.63	13.11°
16	0.03	4.24	0.31	19.24°

A = *Aedes*, T = *Trichocorixa*, E = *Ephydra*. λ_i values were corrected for differences in detectability between the three species[5,14]. Detectabilities were determined in the laboratory with controlled feedings under field temperature and light conditions[5].

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Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons

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Body adiposity is normally maintained within rigid limits[1–3]. Although it is not clear that this regulation fits a strict negative feedback pattern, animals do maintain a relatively constant body adiposity[4]. It has been postulated that this regulation is mediated by some signal which informs centres controlling food intake, probably located in the brain, as to the present state of adiposity[5,6]. The identity of the signal is unknown, but the direct correlation between body adiposity and basal insulin levels in the plasma[7–9], suggests insulin as a possible candidate. This hormone is present in the cerebrospinal fluid (CSF) of many species[10–13], and is a slow integral over time of the level within the plasma[14]. Thus, the level of insulin in the CSF is relatively resistant to short-term plasma fluctuations of insulin. Obese humans have higher levels of CSF insulin than lean controls and the CSF insulin level of both obese and lean humans is reduced proportionately after a prolonged fast[15]. We have therefore postulated[16] that the feedback system responding to body adiposity uses the concentration of insulin in the CSF as a major signal. Additional support for such a role is found in recent reports that insulin receptors are present in several regions of the brain and spinal cord[17–20]. We now present additional evidence for our hypothesis by showing that in baboons the infusion of exogenous insulin into the CSF elicits a reliable and predictable decrease in food intake and body weight.

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To test this hypothesis we have developed a baboon model in which chronic feeding can be monitored in unanaesthetised animals and in which experimental access to the blood and CSF is maintained. Animals were maintained in standard primate restraining chairs for 3–6-week studies during which solutions of sterile buffered salt solution, 'synthetic CSF' (ref. 21), were infused into the animals' lateral cerebral ventricles with or without insulin or glucagon. To do this, an acrylic pod with six cannula guide sleeves was stereotaxically fixed to the skull while the animal was anaesthetised with halothane[22]. The guide sleeves were cut so that their tips would lie at the dorsal surface of the lateral cerebral ventricle, and X rays were used to compare their positions with the plates in Riche[23].

All animals were adapted to a commercially available liquid diet (Ensure, Ross, containing per l approximately 37 g fat, 37 g protein and 145 g carbohydrate with additional vitamins and minerals) which was freely available in stainless steel drinking cans placed on the chairs from 8.30 a.m. to 3.30 p.m. each day. Water only was available from 3.30 p.m. to 8.30 a.m. Food intake was determined by periodic volumetric determinations of remaining diet during the feeding interval, with addition of supplemental diet as appropriate.

To verify that infusions through the cannula actually reached the CSF, we routinely injected each animal with 5 μg of angiotensin II through a cannula 1–2 mm longer than the guide sleeve and determined subsequent water intake. An increase in water intake of more than 50 ml over the 30 m following the injection relative to the water intake on a control day was taken as indicative that the cannula had access to the CSF. The mean water intake over the 30-m interval immediately following the injection of the angiotensin II was 277 ml greater than that on a control day when only the vehicle was injected.

The intraventricular cannulas were infused at a constant rate between 1 and 1.5 ml per day. Infusions of synthetic CSF had no apparent effect on food intake, body weight, basal plasma insulin or glucose levels for prolonged intervals (see Table 1 and Fig. 1). After a control or baseline interval, insulin (bovine/porcine insulin, Lilly) was added to the infusate at doses of 1, 10 or 100 μU per kg per day. A given baboon received each dose of insulin only once, and the order of the various conditions was random. The lowest dose of insulin was chosen because we reasoned that if this was given in a single dose instead of being infused over a 24-h period it would approximately double the concentration of insulin in the CSF. This assumed that the level of insulin within the CSF of the baboon is approximately 25% of the level in the plasma, as it is in other species[10–14], and that an average baboon weighs 15 kg and has a ventricular volume around 1 ml. The increment of CSF insulin achieved with the lowest infusion dose was probably negligible as it was actually infused over a 24-h interval and the half life of insulin in the CSF of the dog is around about 1.5 h (ref. 12).

Results of this procedure are summarised in Table 1, which contains the mean values for a 2-week baseline interval in which only synthetic CSF was infused and analogous mean values for the intervals after with the infusion of insulin or glucagon (Lilly). Figure 1 depicts the daily food intake for these conditions. Although there was considerable daily variation in food intake, there was a significant, dose-related decrease in food intake when insulin was added to the infusate. The data were analysed using non-parametric statistics because there was often heterogeneity of variance between conditions and because not every baboon was used in every condition. For the analyses, a mean value was obtained for each animal over the 14-day baseline period. A second overall mean value was obtained for the interval when the hormone was actually infused. These data were then analysed using an overall Kruskal–Wallis test with individual conditions compared by the Mann–Whitney test[24]; $P < 0.05$ indicated significance. It should be clear that the analysis was very conservative, as the use of some other parameter (for example, a mean of the last 5 days' values in each condition) would have resulted in a larger change, thus giving a greater probability to achieve a significant effect.

There were no reliable differences between baseline parameters for the four conditions (three insulin doses and one glucagon dose). For food intake (Table 1) there was a significant reduction during infusion of both the two larger doses of insulin, and the intake during the largest dose (100 μU per kg per day) was significantly less than that during the intermediate dose

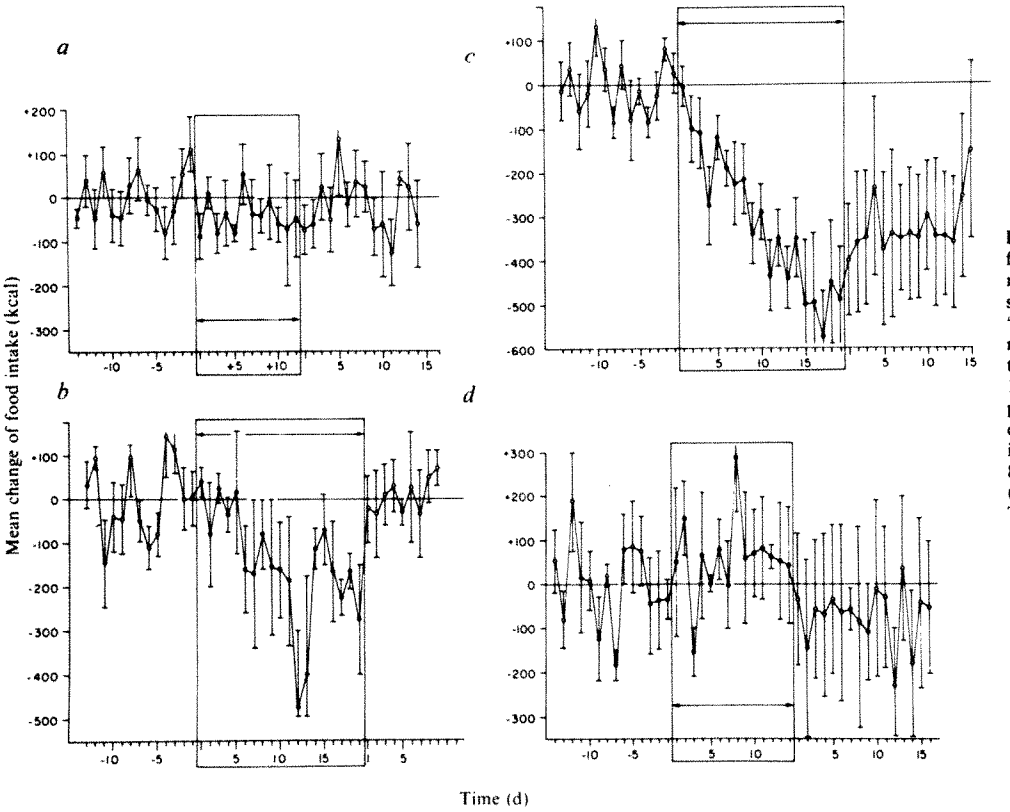


Fig. 1 Daily food intake values for the four experiments. Points and bars represent the mean ±1 s.e.m. Open symbols represent control intervals when 'synthetic CSF' was infused; solid symbols represent intervals when insulin at one of three doses (*a*, 1 μU per kg per day; *b*, 10 μU per kg per day; *c*, 100 μU per kg per day) or glucagon (*d*, 2.35 ng per kg per day) was infused. In *a* the mean baseline intake was 821 kcal d^{-1} ($n = 5$), in *b* 860 kcal d^{-1} ($n = 4$), in *c* 781 kcal d^{-1} ($n = 6$) and in *d* 726 kcal d^{-1} ($n = 3$). Times of hormone infusions are boxed.

Table 1 Food intake, plasma insulin, plasma glucose and body weight for the baseline and hormone-infusion portions of the experiment

	Mean food intake (kcal)*	Mean plasma insulin (μU ml^{-1})*	Mean plasma glucose (mg ml^{-1})*	Mean body weight (kg)*	Final body weight (kg)†
Baseline	821±121	71.5±11.1	86.3±1.9	17.8±2.6	
Insulin (1 μU per kg per day)	786±119	73.8±11.4	85.3±1.3	17.8±2.7	17.8±3.0
Change (n = 5)	−35±12	+2.3±5.5	−1.0±2.4	+0.04±0.08	−0.01±0.08
Baseline	860±135	65.1±11.6	84.3±1.2	18.8±3.3	
Insulin (10 μU per kg per day)	702±97	66.1±8.4	86.0±2.7	18.5±3.2	18.4±3.0
Change (n = 4)	−158±57‡	+1.0±6.0	+1.7±3.9	−0.28±0.15	−0.37±0.12‡
Baseline	781±99	67.2±3.9	81.0±4.0	17.8±2.3	
Insulin (100 μU per kg per day)	462±99	63.5±4.4	82.5±1.5	17.4±2.2	17.0±2.4
Change (n = 6)	−319±45§	−3.9±0.5	+1.5±2.5	−0.33±0.12‡	−0.81±0.09‡
Baseline	726±104	46.3±2.1	87.5±4.5	14.5±1.2	
Glucagon	788±60	52.9±10.3	87.5±2.5	14.6±1.3	14.7±1.6
Change (n = 3)	+62±61‖	+6.6±8.3	0±2.0	+0.10±0.06‖	+0.19±0.04

* These values represent an average (mean±s.e.m.) for the entire infusion interval.
† These values are those of the final day of hormone infusion and are indicative of the total weight change achieved.
‡ Significantly different than the analogous value during the lowest dose of insulin.
§ Significantly different than the analogous value during the intermediate dose of insulin.
‖ Significantly different than the analogous value during the highest dose of insulin.

(10 μU per kg per day). The food intake during the glucagon infusion did not differ significantly from that during the baseline period, nor from that during the infusion of the smallest dose of insulin. It was significantly greater than the intake during the infusion of the largest dose of insulin. With respect to body weight, only the largest infusion of insulin resulted in a significant decrease relative to the baseline or to the smallest dose. The body weight during the glucagon infusion was not consistently different from that during the baseline but significantly larger than that obtained during the infusion of the largest dose of insulin. There were no differences in plasma insulin (as determined by immunoassay)[25] or plasma glucose (as determined by the glucose oxidase method) during any hormone infusion interval relative to control. There was a significant dose-related reduction in body weight, but no reliable change in plasma insulin or glucose during these intervals (Table 1), suggesting that leakage of infused insulin from the CSF to the blood was minimal.

The weight losses at the end of the sessions during which the two larger insulin doses were infused (see last column in Table 1) were approximately what one would expect based on the decrease in food intake. If one assumes that 1 g of stored fat yields about 7 kcal (ref. 1), the mean daily decrease of 158 kcal for 19 days (based on the figures for the 10 μU per kg per day dose) would be expected to cause a mean decrease of 429 g of body weight. The actual decrease was 374 g. Similarly, the expected decrease in weight in animals given the largest insulin dose for 19 days would be 866 g, whereas the actual weight loss was 812 g.

At the end of an experimental infusion session in which food intake was depressed, food intake rapidly returned towards the baseline control level. However, the rebound was not complete following the infusion of the largest dose of insulin even after 2 weeks (see Fig. 1).

Glucagon was infused as a peptide control at the same molar dose as the highest effective dose of insulin. As shown in Table 1 and Fig. 1, glucagon infusion at this dose had no consistent effect on food intake, body weight, plasma insulin or plasma glucose. If anything, there was a slight tendency for the baboons to increase their food intake. This was most apparent as a rebound decrease in food intake after the end of the glucagon infusion (Fig. 1), although this result was not statistically significant. However, it is clear that the suppression of food intake observed when equimolar amounts of insulin were infused did not occur with glucagon. This control therefore makes some nonspecific illness-related explanation unlikely, especially when one considers that glucagon, when given peripherally, reportedly makes animals ill and causes them to eat less[26,27].

We have interpreted these results to support the theory[16] that CSF insulin levels may serve as a signal to inform the brain of the present state of adiposity. It may well be that other, analogous systems exist in which CSF-borne peptides influence behaviour. For body weight regulation, the observed system closes the feedback loop between the brain and the endocrine pancreas[28,29]. Independently of the theoretical interpretation, there are potential practical implications of these findings. We have found that we can reduce the food intake and body weight of baboons by altering the amount of a hormone already present in the CSF. It will be important to determine if this system is operative in man and if there are possible therapeutic implications for human obesity.

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Nerve sheaths and motoneurone collateral sprouting

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When disease or injury causes partial loss of innervation from a muscle, the remaining axons sprout and form new connections to the denervated muscle fibres[1]. Sprouting can occur in two ways: from axon terminals (terminal sprouting) or from the intramuscular axons themselves, probably from the nodes of Ranvier (collateral sprouting)[1,2]. Terminal sprouting has been induced experimentally using various methods, including partial denervation[2], nerve conduction block[3] and nerve transmission block[4,5]. A common factor in the induction of terminal sprouting seems to be changes in the surface membrane of muscle fibres[6,7]; these changes and terminal sprouting are prevented by direct stimulation of the muscle[5]. Collateral sprouting has been induced only by partial denervation and is not prevented by direct stimulation[5]. This has been taken as evidence[6] for an earlier suggestion[2] that products of nerve or axon degeneration may be a direct stimulus for collateral sprouting. We report here that axon degeneration products alone are probably not the stimulus for collateral sprouting.

Collateral sprouting in response to partial denervation was examined in a mouse hind limb muscle, tensor fascia latae (TFL), which is normally innervated by 11 axons from lumbar spinal nerves L4 and L5. Mice (15–20 g body weight) were anaesthetised intraperitoneally with chloral hydrate (0.1 ml of 5% solution per 10 g body weight), and partial denervation of TFL was achieved by cutting and redirecting the proximal stump of L4 into the peritoneal cavity. This operation results in the degeneration of a variable number of TFL axons, but usually nine. Muscles were exercised up to 30 days after the operation, stained by the combined silver and cholinesterase method of Namba *et al.*[8], mounted whole between two coverslips and examined in the light microscope under a ×100 oil immersion objective. By this method endplates, axons, axon sprouts and perineurial sheaths were visualised throughout the entire muscle. The lengths of sheaths, axons and sprouts were measured using a calibrated 10×10 square graticule in the microscope eyepiece. Sprouts are clearly distinguishable from the pre-existing terminal axons by two criteria. First, the diameter of sprouts and pre-existing axons are significantly different (0.47 ± 0.10 μm, $n = 38$ and 1.79 ± 0.39 μm, $n = 95$, mean ± s.d., respectively). Second, sprouts have uniformly smooth profiles while pre-existing terminal axons possess characteristically gnarled profiles (Fig. 1).

The nerve of TFL branches within the muscle to form smaller bundles of axons (intramuscular nerves) and finally single terminal axons which contact individual muscle fibres. There are no sprouts in muscles removed 1 day after partial denervation. Extensive axonal degeneration is visible within the perineurial sheaths of the intramuscular nerves, some of which contain surviving intact axons. Denervated terminal axon sheaths arise from intramuscular nerve branches and terminate on cholinesterase-stained endplates. Muscles removed from animals two or more days after partial denervation show fine collateral sprouts, which reach denervated muscle fibres by growing down denervated sheaths. Not all the sprouts observed reached cholinesterase-stained endplates. Some (21%, $n = 190$) end in bulbous enlargements similar in appearance to growth cones described by Ramon Y Cajal[9] in silver-impregnated developing axons. These sprouts which end within perineurial sheaths are most common in muscles partially denervated between two and five days before excision.

Sprouts arise from intact axons close to points at which denervated sheaths branch from partially denervated intramuscular nerves (Fig. 2). In four muscles partially denervated for less than 9 days sprout sites are ~22 μm from the branch points (22.4 ± 1.8 μm, $n = 77$). In two muscles partly denervated for 16 and 30 days, sprouts arise ~38 μm from the branch points (38.1 ± 43.9 μm, $n = 62$). Sprouts are absent from quite long segments of intramuscular nerve (400–600 μm). If axon degeneration products were the sprouting stimulus, sprouts would be expected throughout these segments, because they

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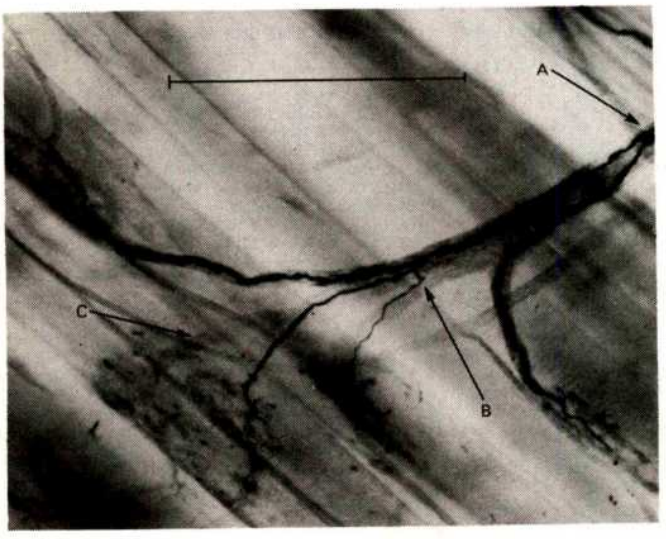


Fig. 1 Light micrograph of combined silver and cholinesterase-stained TFL muscle. The muscle was partially denervated 8 days before excision. Perineurial sheaths enclose two pre-existing terminal axons which arise at arrow A. Cholinesterase enzyme product is visible as speckled deposits over terminal arborisations. A fine collateral sprout arises at arrow B and follows a sheath to a nearby endplate. A denervated sheath (arrow C) arises near B. The axon branch between sprout B and denervated sheath C is one of a small number of profiles (9%, $n = 741$) with a diameter intermediate between that of sprout and pre-existing axon. It may be either a sprout in the process of maturation or an unusually thin pre-existing axon; these are excluded from all sprout statistics. Scale bar, 100 μm.

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contain degenerating axons for several days following partial denervation. The degenerating axons are not separated from the intact axons by septa[10] or other diffusion barriers which might prevent access of degeneration products to intact axons (unpublished observations of electron micrographs). The absence of sprouts is not due simply to the absence of potential sprout sites, probably nodes of Ranvier, which occur frequently in these segments (internodal distance = 163 ± 61 μm, $n = 31$, from three muscles). Moreover, the sprout-free segments of nerve are

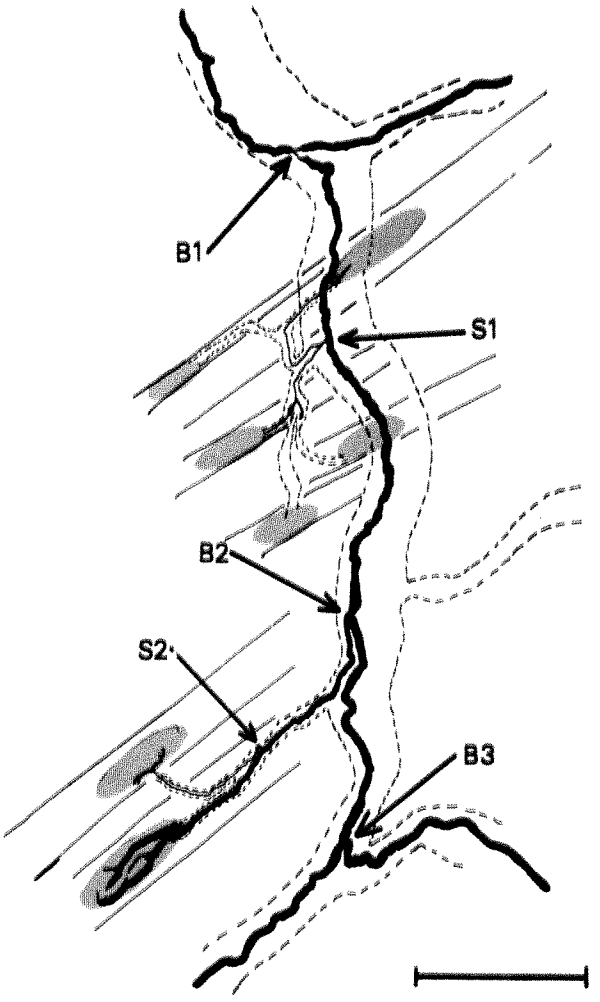


Fig. 2 Camera lucida tracing of a segment of intramuscular nerve from a TFL muscle excised and stained 4 days after partial denervation. Perineurial sheaths enclose degenerating axons (not drawn) and a single intact axon with branch points at B1, B2 and B3. Sprouts arise at S1 and S2 and innervate endplates (shaded areas). A terminal axon arising at B2 also innervates an endplate within the field shown. Note that there is no sprouting at the branch points. In other regions of this muscle, the nodes of Ranvier at branch points are also sprout sites. Scale bar, 100μm.

flanked by denervated sheaths containing sprouts, making it unlikely that these segments are for some reason refractory to a sprouting stimulus from degenerating axons.

Denervated terminal axon sheaths become sprout innervated in a sequential manner. We have measured the lengths of sprout-containing sheaths from the endplate to the point where the sprout arises in the intramuscular nerve. In some cases where the sprouts ended in a 'growth cone' short of the cholinesterase-stained endplate we measured the entire distance from the point of sprout outgrowth along the sheath to the endplate. We found that the shortest denervated sheaths are the first to become innervated by sprouts, as shown in Fig. 3.

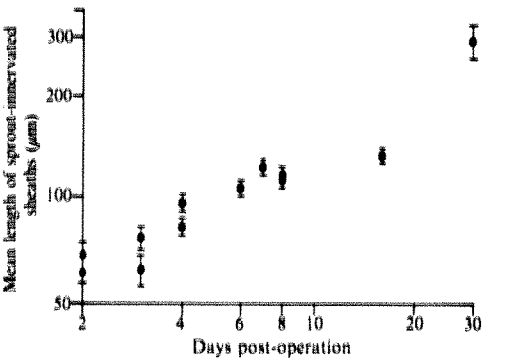


Fig. 3 Mean length of sprout-innervated sheaths plotted as a function of time after partial denervation. Each point represents one muscle and shows the mean ±s.e.m. for between 21 and 68 sheaths. Mean pre-existing terminal axon length for the 12 muscles in this figure is 130 ± 8 μm (mean ± s.d.).

These results do not seem to be compatible with a mechanism in which sprouts are initiated simply by the products of nerve or axon degeneration. At least two classes of mechanism for collateral sprout initiation are consistent with our findings. First, the sprouting stimulus might be a diffusable substance in the extracellular space which reaches intact axons either directly across denervated sheaths or via the terminal ends of denervated sheaths. Second, the stimulus might arise from changes in either the denervated Schwann or perineurial sheath cells developing first near the terminal ends of the sheaths, and subsequently spreading back towards the intramuscular nerves containing intact axons.

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Teratogenic drugs inhibit tumour cell attachment to lectin-coated surfaces

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Interactions between embryonic cells are generally thought to have a central role in the control of development[1]. When these morphogenic interactions are interrupted by either physical intervention[2] or genetic defects[3], normal development is impaired. In accord with these experiments, specific interactions between embryonic cells have been demonstrated in several *in vitro* systems. Many investigators have described homotypic aggregation of chick embryo cells[4–6], and heterotypic specificity has also been described[9]. Because of the importance of morphogenic cell–cell interactions in development it follows

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that agents that interfere with these interactions, regardless of the interference mechanism, are potential teratogens. Here we have used a simple *in vitro* cell to surface recognition system in an attempt to screen for potential teratogens. We have found a very high correlation between inhibitory activity in the *in vitro* assay and reported teratogenic activity in human or animal studies. This suggests that many teratogenic agents may act by interfering, in an as yet unknown way, in normal cell to cell interactions.

In this system, tumour cell attachment to concanavalin A-coated plastic surfaces, mediated by a cell-surface carbohydrate–concanavalin A receptor interaction, has been used to mimic some features of the complex interactions to be expected during development. Tumour cells are useful because of their availability in large, homogeneous quantities and ease of preparation. In addition, they have long been known to have properties in common with cells of embryonic origin[10]. Of particular relevance is the observation that although normal fibroblasts attach poorly to surfaces derivatised with lectins (ref. 11 and A.G.B., unpublished observations), transformed fibroblasts, tumour cells (refs 11–13 and A.G.B., unpublished observations) and some embryonic cells (P. Spear, J. L. Wang and G. M. Edelman, unpublished, but cited in ref. 25), attach to these surfaces. Both embryo cell aggregation and tumour cell attachment to lectin-coated surfaces require energy[6,11]. Thus, it is plausible that the attachment of tumour cells to lectin-coated surfaces can serve as a simple *in vitro* model for the morphogenic interactions observed *in vivo*.

Tumour cell attachment to lectin-coated surfaces was quantified by using 1.25-cm diameter polyethylene disks and ascitic mouse ovarian tumour (MOT)[14] labelled with tritiated thymidine *in vivo*. We have found that the tumour cells attach to lectin-derivatised disks rapidly, within 5 min at room temperature. To assay for the inhibitory activity of a drug, tumour cells (1 ml at 5×10^6 per ml) were incubated with the drug, usually for 30 min at 37 °C, poured into a 35-mm polycarbonate Petri dish and several disks submerged into the suspension. As dead cells do not attach to lectin-coated surfaces the drug concentrations used here were kept below overtly toxic levels as determined by trypan exclusion. After a 20 min incubation at room temperature the disks were removed with fine forceps, washed in saline and counted in a scintillation counter. An example of the inhibitory activity of several anaesthetics and tranquilisers is shown in Fig. 1. Similar concentrations of these drugs have been reported to inhibit lymphocyte capping in similar conditions[15]. Of the five drugs shown in Fig. 1, four are known to cause congenital malformations *in vivo*[16]. We have been unable to determine whether lidocaine is a teratogen.

Curves similar to those of Fig. 1 have been generated for 55 drugs with known teratogenic properties. To characterise the inhibitory activity of a drug we have found it useful to determine the ID_{50}—the dose of drug that inhibits 50% of tumour cell attachment. Table 1 lists these 55 drugs according to their inhibitory properties. Of drugs found to inhibit attachment, 31 of 33 (94%) have been reported to be teratogenic *in vivo*. Note the wide range of agents which inhibit attachment; these include

Table 1 Inhibition of tumour cell attachment to concanavalin A-derivatised polyethylene disks

Inhibitory drug				Non-inhibitory drug			
	ID_{50}	Teratogen	Ref.		Maximum concentration	Teratogen	Ref.
Aspirin	14 mM	+	16	Colchicine*	5 mM	+	16
Phenylbutazone	0.12 mM	+	17				
Procaine	11 mM	+	16	Chloroform	33.5 mM	−	18
Warfarin	0.8 mM	+	16	Heparin	5 U ml^{-1}	−	16
Caffeine	35 mM	+	16				
Diphenylhydantoin	0.84 mM	+	16				
Diazepam	0.88 mM	+	16				
Meprobamate	2.3 mM	+	16				
Chlordiazepoxide	0.17 mM	+	16				
Trifluoperazine	0.1 mM	+	16	Thalidomide*	1 mM	+	16
Chlorpromazine	0.1 mM	+	16				
Pentobarbital	1.4 mM	+	16				
Dexamethasone	0.2 mM	+	16	Hydrocortisone-21-hemisuccinate*	1 mM	−	16
Prednisolone-21 sodium succinate	12 mM	+	16	Cortisone acetate	0.3 mM	+	16
Phenylalanine	90 mM	+	16	Lysine	45 mM	−	16
Retinoic acid*	0.4 mM	+	16	Cysteine	75 mM	−	16
				Thiamine	2.5 mM	−	16
				Ascorbic acid	75 mM	−	16
				Ouabain	4.5 mM	−	16
Cytochalasin B*	0.69 mM	+	23	Ammonium chloride	75 mM	−	16
Dinitrophenol	0.1 mM	+	16	EDTA	10 mM	−	16
DMSO	0.9 M	+	16	Monosodium glutamate	150 mM	−	19, 20
Tween	0.38 mg ml^{-1}	−	16	Propylene glycol	0.1%	−	16
Linoleic acid	0.053 mM	−	24	Carboxymethyl cellulose	9 mg ml^{-1}	−	16
				Trypan blue	0.83 mM	+	21
Penicillin G	17 mM	+	22	Streptomycin	16 mM	−	25
Chloramphenicol	2.8 mM	+	16				
Nalidixic acid	0.5 mM	+	16				
Triton X-100	0.015 mM	+	16				
Ethanol	0.54 M	+	16				
Busulfan	1.3 mM	+	16	Actinomycin D	0.2 mM	+	16
BCNU†	2.3 mM	+	16	Methotrexate	2.2 mM	+	16
				5-Fluorouracil	1.9 mM	+	16
				Vinblastine	1.1 mM	+	16
Griseofulvin	0.14 mM	+	16				
Diethylstilboestrol†	0.11 mM	+	16				
Oestradiol†	0.11 mM	+	16				
Adrenaline HCl	0.46 mM	+	16				
Noradrenaline HCl	0.24 mM	+	16				

Drugs were tested for inhibition of attachment as described in the text and in Fig. 1 legend. In nearly all cases incubation with drugs was for 30 min at 37 °C. The assignment of *in vivo* teratogenic activity was based on the reference cited. +, Teratogenic *in vivo* according to cited ref.; −, non-teratogenic, according to cited ref. The maximum concentration tested was limited by solubility of the drug in phosphate-buffered saline or by its toxicity as judged by trypan blue exclusion.

* Drug dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO did not exceed 0.3 M, a non-inhibitory concentration.

† Drug dissolved in ethanol. The final ethanol concentration was not inhibitory.

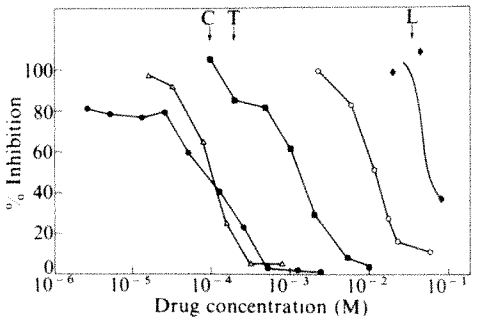


Fig. 1 Inhibition of MOT cell attachment to concanavalin A-derivatised disks. Disks were cut from 15-ml polyethylene with a 1.25 cm diameter die and derivatised in a solution of 0.1 mg ml^{-1} concanavalin A and 2.5% glutaraldehyde overnight. The disks were washed in buffer and stored in 0.3 M glycine. Just before use they were again washed in buffer. The surface density of concanavalin A receptors on disks prepared in this manner has been estimated by tritium-labelled glucose binding to be 3×10^{12} receptors cm^{-2}. In this experiment ^{3}H-labelled MOT cells at 5×10^6 per ml were incubated with the indicated concentration of drug for 15 min at room temperature and attachment to the disks determined as discussed in the text. Triplicate samples were assayed, averaged and the data expressed in terms of attachment of untreated cells. ●, Chlorpromazine (Thorazine); △, trifluoperazine (Stelazine); ■, pentobarbital (Nembutal); ○, procaine (Novocain); ◆, lidocaine (Xylocaine). The labelled arrows indicate the concentrations of chlorpromazine (C), trifluoperazine (T) and lidocaine (L) required to inhibit IgG-mediated lymphocyte capping[15].

antipsychotics, anti-inflammatory agents, oestrogens and steroids, fatty acids, anaesthetics, tranquilisers, an anticonvulsant, an alkylating agent, an antifungal antibiotic, an anticoagulant, detergents and an alcohol.

Although inhibition of attachment corresponds to teratogenic activity *in vivo*, failure to inhibit does not correlate as well with lack of teratogenic activity. Table 1 indicates that of 22 drugs found to be non-inhibitory, 14 (64%) were also non-teratogenic *in vivo*. The remaining 8 (36%) thus constitute false negatives (teratogens which do not inhibit attachment). Several of these false negatives are known inhibitors of macromolecular synthesis and would not be expected to have dramatic effects on the cell surface during the 50 min of the assay. Other agents, notably Thalidomide, are believed to be teratogenic by virtue of metabolic products rather than the parent compound[16]. As the current assay system does not include a metabolic activation system, drugs requiring activation would not be expected to inhibit attachment. Recent experiments have shown that treatment of Thalidomide with liver microsomes yields a metabolite which inhibits attachment.

These data suggest that the inhibition of attachment *in vitro* may be useful in the prediction of teratogenic activity *in vivo*. If the five inhibitors of macromolecular synthesis and assembly are excluded, 44 of 50 tested drugs (90%) correctly predict *in vivo* teratogenic response. It is unlikely that the correspondence shown here is completely fortuitous. It is more likely that many teratogens exert their effect by interfering with normal morphological interactions and that these agents also interfere by a similar but unknown mechanism with the energy-dependent tumour cell to lectin surface interaction. However, some of these agents, such as BCNU and busulfan, are probably teratogenic because of their toxicity rather than their interference with cell to cell interactions.

Although the data reported here concern attachment of MOT cells to concanavalin A-derivatised surfaces, we have found identical attachment characteristics with cells from Ehrlich ascites tumour and with cells derived from solid tumours by collagenase digestion. Attachment to surfaces derivatised with lentil lectin or wheat-germ agglutinin was identical to that observed with concanavalin A. Attachment characteristics do not seem to depend on the nature of the plastic surface, as Teflon, Mylar, polycarbonate and acrylic surfaces derivatised with concanavalin A had characteristics identical to the polyethylene surfaces. Scanning electron micrographs of attached cells indicate that attachment is mediated exclusively by numerous filopodia. During the 20-min attachment period, gross spreading of the tumour cells does not occur. However, there is some preliminary evidence that the filopodia extend and arborise during this interval.

If the inhibition of tumour cell attachment to lectin-coated surfaces continues to correlate well with teratogenicity, this inexpensive assay system may prove useful in the rapid identification of potentially teratogenic environmental agents and drugs.

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Antibody-mediated enhancement of Flavivirus replication in macrophage-like cell lines

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Interactions between animal viruses and antiviral antisera may exceptionally result in an apparent increase in viral infectivity[1–3]. Halstead and coworkers[4–6] demonstrated enhanced replication of dengue virus (a Flavivirus, family Togaviridae) in human or simian peripheral blood leucocytes carrying Fc receptors at subneutralising concentrations of anti-dengue antibody. We have used three continuous cell lines[7–9] which express macrophage markers to explore the mechanism of this phenomenon. Dengue virus failed to replicate in these cells, but West Nile virus, another Flavivirus, replicated in all three, and we were able to demonstrate reproducibly 50–100-fold enhancement of virus yields in the presence of Flavivirus antisera, the effect also being directly demonstrable in P388D$_1$ cells by increased numbers of virus-induced plaques. The phenomenon of antibody-dependent enhancement of viral replication is not unique to dengue virus, and may have far wider relevance in other viral infections.

P388D$_1$ cells were used[7]. This was continuous line derived from a methyl cholanthrene-induced mouse tumour, which shows a number of macrophage characteristics, for example macrophage-like morphology, immune phagocytosis, Fc and C3 receptors, production of lysozyme, effector functions in antibody-dependent cell mediated cytotoxicity assays, and the absence of surface immunoglobulin[10,11]. Also a second mouse cell line, J774.1 (ref. 8) and a continuous human histiocytic lymphoma cell line, U937 (ref. 9).

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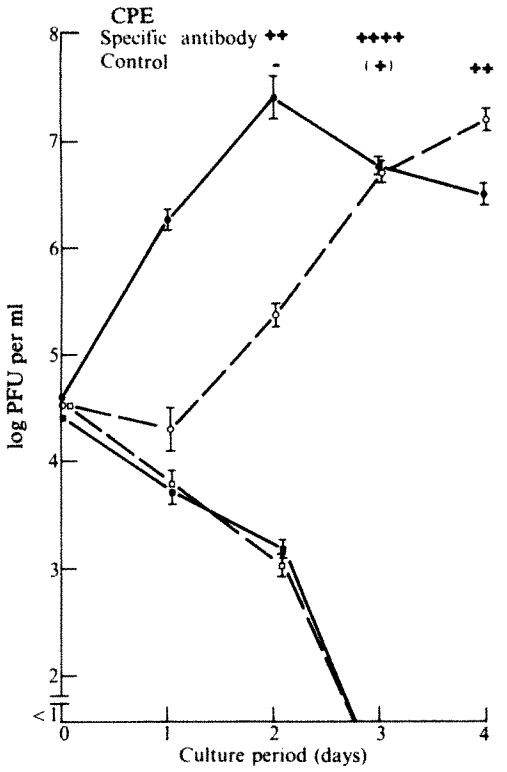


Fig. 1 Effect of specific antibody on West Nile virus yields in $P388D_1$ cells. $P388D_1$ cells (1.3×10^6) in 60 mm Falcon tissue culture dishes were infected with West Nile virus (multiplicity of infection 0.02). Rabbit antibody to normal mouse brain or West Nile virus infected mouse brain at a final dilution of 1:1,000 was added to respective culture dishes. Aliquots of supernatant medium were removed and titrated for virus infectivity in PS cells at days 0, 1, 2 and 3 post-infection. Control tissue culture dishes without cells were set up to estimate thermal inactivation of virus. Each point represents the mean of six titrations, and the 95% confidence limits of the mean is denoted. Infectious virus titre in $P388D_1$ cells in presence of antibody to: ○- - -○ normal mouse brain; ●——● WNV-infected mouse brain. Thermal inactivation of West Nile virus (identical experiment carried out in the absence of $P388D_1$ cells) in presence of antibody to: □ - - -□ normal mouse brain; ■——■ WNV-infected mouse brain. The degree of cytopathic effect in the $P388D_1$ cell monolayer is represented:—no CPE; (+) less than 10% of cells affected; +10–25% cells affected; ++ 25–50% cells affected; +++ 50–75% cells affected; ++++ 75–100% cells affected.

$P388D_1$ cells were grown in MEM-Alpha medium (Gibco-Bio-cult) with 10% heat inactivated fetal calf serum (Sera-Lab) and antibiotics. West Nile virus (WNV) (Egypt 101) stock was a 10% homogenate of infected suckling mouse brain stored in aliquots at −70 °C (infectious titre 2×10^7 plaque forming units (PFU) per ml). A 28-day rabbit antiserum raised against WNV was used in experiments where antibody was added. Titrations of virus infectivity were done using pig kidney PS cells[12]. Figure 1 shows the infectious virus yield from $P388D_1$ cells infected with WNV at a multiplicity of infection of 0.02; in the presence of anti-WNV antibody at subneutralising concentrations this was increased 100-fold in comparison with control cultures. The virus yield from antibody enhanced cultures of $P388D_1$ cells peaks on the second day post-infection, when there were no viable cells left to support viral replication. Yields from control cultures attain the same final virus titre as 'antibody-enhanced' culture plates, but about two days later.

In the presence of antibody, marked cytopathic effect was observed on the second post-infection day which progressed to total destruction of the cell sheet by day 3. In contrast, culture dishes infected in identical conditions without antibody, showed a mild cytopathic effect on day 3 which progressed to total cell destruction on post-infection day 5. Control experiments showed that the effect of the anti-WNV serum was not simply to protect WNV from thermal inactivation. Both the 'enhancement' of replication and the earlier cytopathic effect caused by anti-WNV antibody were reproducible even when unabsorbed virus was removed after the initial infection by two cycles of washing in phosphate-buffered saline.

We next demonstrated antibody-mediated enhancement of WNV plaque formation in $P388D_1$ cells under carboxymethyl cellulose overlay. Figure 2 shows the dramatic increase in the number of plaques resulting from the inclusion of specific antiserum at subneutralising concentrations (final dilution 10^{-3}) in the culture medium. This technique was used to study the ability of a range of antisera at different dilutions to demonstrate antibody-mediated enhancement of plaque formation by WNV (Table 1). Using the anti-WNV serum, it was noted that high concentrations of antibody (up to a final dilution of 10^{-2}) result in neutralisation of plaque formation. At subneutralising concentrations (final dilution $10^{-3} - 10^{-4}$) a 30–40-fold increase in plaque number occurs, and at higher dilutions of antiserum, the plaque counts approach control numbers. Appropriate concentrations of antibody against other relatively closely related (dengue) or more distantly related (Kyasanur Forest disease) Flaviviruses were capable of enhancing WNV, but antisera against the antigenically unrelated Alphavirus, Semliki Forest virus, or against normal mouse brain did not affect the plaque count. This suggests that antibodies to group-specific antigenic determinants are capable of producing enhancement.

Experiments with the cell lines J774.1 and U937 infected with WNV confirm the increased yield shown in $P388D_1$ cells in the presence of specific antiserum, but plaque formation was not possible in these two cell lines. In contrast, virus yields from PS cells (an epithelial cell line of pig kidney origin lacking Fc receptors[13]) were not significantly altered by the presence of 'enhancing' concentrations of antibody (unpublished observations). However, plaque formation by WNV in PS cells seems to be marginally increased by appropriate concentrations of antibody (1.7-fold over control plaque members $P < 0.001$–unpublished observation). In experiments with human peripheral blood leukocytes, WNV shows antibody mediated

Table 1 Enhancement of West Nile virus plaque formation by homologous, cross reacting and non-cross reacting antisera.

Antiserum	'Enhancement factor' at different antibody dilutions						Antibody titre vs. West Nile virus	
	1/25	1/100	1/1000	1/10,000	1/100,000	Control	H.A.I.	PRNT
Pre immune serum	1.4	0.7	1.1	0.7	1.1	1	<10	<10
Anti West Nile immune serum	0	1.0*	20	18	1.2	1	320	64
Anti Dengue type 2 immune serum	12	19	0.7	0.8	1.1	1	20	<10
Anti Kyasanur forest disease immune serum	9	2	0.9	1.2	0.8	1	40	<10
Anti Semliki Forest virus immune serum	1.1	0.8	0.9	0.9	1.2	1	<10	<10

The technique used is as for Fig. 2. The numbers represent the 'enhancement factor' at a given dilution of antibody as calculated by mean PFU in presence of antibody dilution/mean PFU in absence of antibody. The virus input was adjusted to give 5–10PFU in control plates (viz in the absence of antibody). * Plaques were smaller than control. HAI = haemagglutination-inhibition antibody titre. PRNT = plaque reduction neutralisation titre.

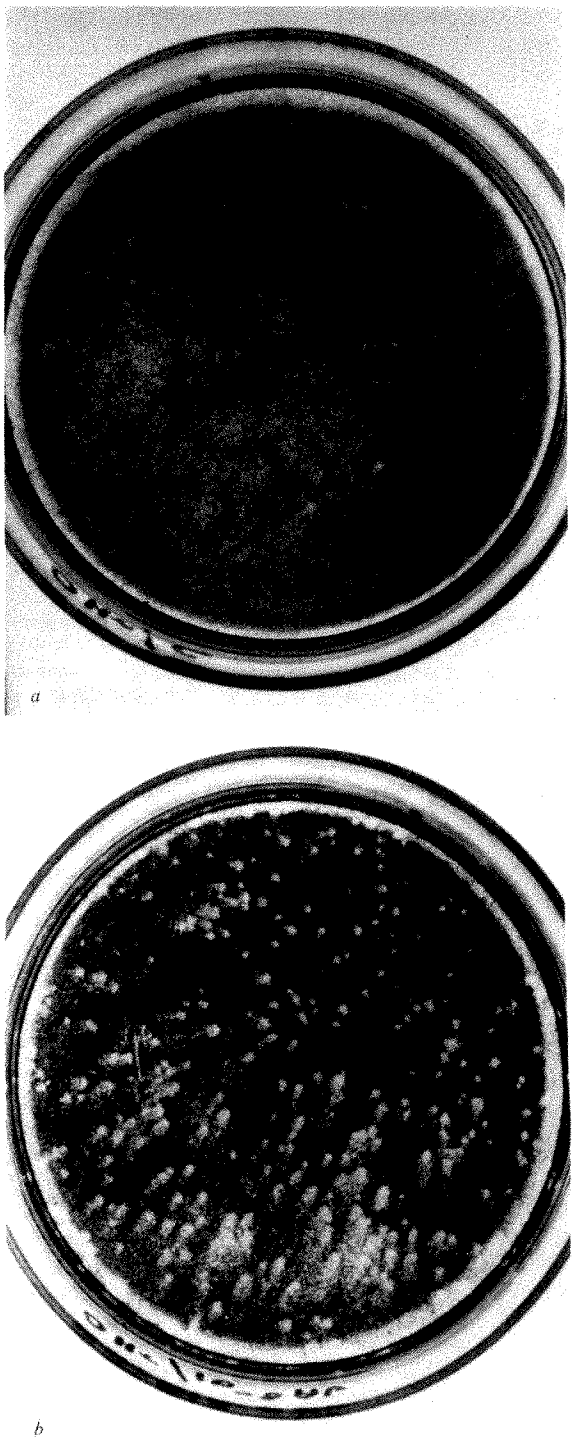


Fig. 2 Enhancement of West Nile plaque formation in P388D$_1$ cells by homologous antibody. 1 ml of virus dilution was mixed with an equal volume of *a*, medium; or *b*, specific antibody at 1 : 1,000 final dilution in 60-mm tissue culture dishes (Falcon). P388D$_1$ cells (1 ml) (1.8×10^6 cells ml^{-1}) was added and culture dishes incubated for 4 h in a CO_2 incubator at 35 °C. Overlay medium (3 ml) containing carboxymethylcellulose (1.5%) was added and culture dishes reincubated for 3 days. At this time the cell monolayer was stained with 1% naphthalene black.

enhancement of virus replication (unpublished observation) thus suggesting that dengue virus is not unique in this respect.

The mechanism of antibody mediated enhancement of viral replication is still open to speculation. The work of Hawkes[1], Halstead and coworkers[4,5] and our own, has been done on cell populations containing mononuclear phagocytic cells (chick embryo fibroblasts have been shown to contain large numbers of phagocytic cells[4]). Fc receptors on such cells may promote the entry of infectious immune complexes formed in the presence of subneutralising concentrations of antibody. Whether antibody directed against a particular antigenic determinant of Flaviviruses is capable of both 'enhancement' and neutralisation depending upon concentration, or whether antibodies of certain specificities are incapable of neutralisation and form 'infectious immune complexes' is uncertain.

The universality of this phenomenon among other virus groups remains to be investigated. Preliminary experiments indicate that antibody also enhances Alphavirus replication in the P388D$_1$ cells (unpublished observations). We believe that the experimental model described could contribute to our understanding of the pathogenesis of diseases such as dengue haemorrhagic fever and other viral infections.

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Microtubule organisation in fibroblasts from dystrophic chickens and persons with Duchenne muscular dystrophy

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In spite of many attempts to correlate the expression of the Duchenne muscular dystrophy (DMD) genotype with defined phenotypic changes in both muscle and non-muscle cells from affected individuals[1–4], the molecular basis of this X-linked genetic defect has not yet been identified. However, Shay and Fuseler reported recently[5] that interphase cells derived from the muscle of newborn dystrophic chickens had greatly diminished cytoplasmic microtubule complexes, and they speculated that this might be due to a specific defect in the microtubule-organising centres. Insofar as microtubules are a major component of the intracellular cytoskeleton[6], a network thought to interact with the cell-surface membrane[7], the possibility that a microtubule defect might explain the membrane alterations associated with muscular dystrophies[1–4] makes this finding extremely interesting. To assess the generality of this finding with respect to chicken dystrophy and to extend the approach to DMD, we used indirect immunofluorescence with an antiserum to purified brain tubulin to examine the organisation of microtubules in cells from the muscle of dystrophic chickens and in skin fibroblasts from persons with DMD. Unlike Shay and Fuseler[5], we find no lack of a microtubular network in cells derived from cardiac or skeletal muscle of chickens carrying the dystrophic gene (*am*). Furthermore, our examination of fibroblasts from persons with DMD revealed a similar extensive network of microtubules in each case.

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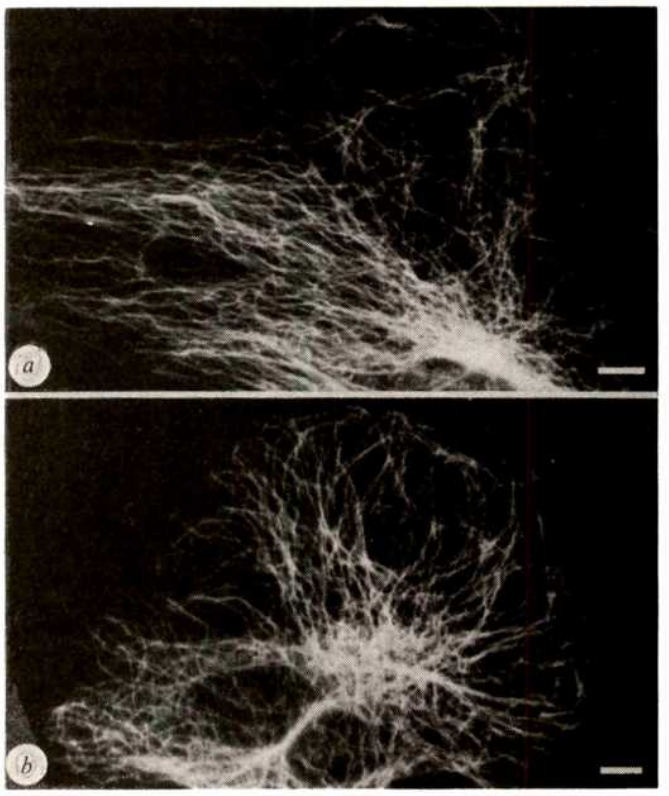


Fig. 1 Fibroblasts prepared by trypsin digestion from 1-d-old dystrophic (*a*) and normal (*b*) chicken muscle stained with antiserum to tubulin. In both cell types a similar cytoplasmic network of microtubules is clearly evident. Scale bar, 5 μm.

To obtain dystrophic chicken cells, we used chickens of the Connecticut line[8]. These birds carry the dystrophic gene (*am*) and are characterised phenotypically by the onset of clinical symptoms 2–3 weeks after hatching[8]. White Leghorn chickens were used as controls. Cells were prepared in one of two ways. (1) A single cell suspension was prepared from trypsinised skeletal muscle tissue[9] and grown to confluency in α-minimal essential medium (MEM) containing 10% fetal bovine serum (FBS). Cells were examined after a single subculture in the same medium. (2) Small pieces of cardiac and skeletal (leg) muscle were placed on 22-mm glass coverslips, allowed to attach for 10 min, and then 2 ml of either α-MEM or CMRL 1066 (GIBCO) culture medium containing 10% horse serum and 5% FBS was added, according to the method of Shay and Fuseler[5]. Cells from these muscle explant cultures were fixed for immunofluorescence after 7 d in culture. Fibroblasts were established in culture from skin biopsies of persons with DMD (three males, 4–5 yr old) and controls of the same sex and approximate age (two males, 4 and 6 yr old). Cells were recovered from storage in liquid nitrogen, and grown at 37 °C under 5% CO_2 in α-MEM supplemented with 15% FBS and antibiotics (100 U per ml penicillin, 100 μg per ml streptomycin). Cells were subcultured routinely every 2–3 weeks and examined by immunofluorescence between the fifth and ninth subculture.

For immunofluorescence, cells were fixed either with methanol followed by acetone at −20 °C (refs 10 and 11), or with methanol alone after treatment with 0.5% Triton X-100 (ref. 12). Fixed cells were incubated with a previously characterised rabbit antiserum to electrophoretically purified pig brain tubulin[10,11] diluted 1:30 in phosphate-buffered saline (PBS) for 40 min at room temperature, washed three times in PBS, and incubated for 30 min with a 1:5 dilution of fluorescein-conjugated goat IgG directed against rabbit IgG (Hyland Diagnostics). Cells were then washed three times in PBS and mounted in 50% glycerol in PBS, *p*H 7.8.

When dystrophic chicken cells, cultured from trypsin-digested muscle or from muscle explants were examined, an extensive network of cytoplasmic microtubules was always seen in more than 98% of the cells (Table 1). Figure 1 shows fibroblasts prepared by trypsin digestion of skeletal muscle from 1-d-old birds. In both dystrophic (Fig. 1*a*) and normal (Fig. 1*b*) cells, the network of cytoplasmic microtubules is clearly evident. Figure 2 shows portions of two cells from explant cultures of dystrophic (Fig. 2*a*) and normal (Fig. 2*b*) skeletal muscle cultured from 15-d-old birds. Again, no obvious differences in the organisation of microtubules were evident. Similar results were obtained independent of the age of the bird from which the cells were cultured, whether skeletal or cardiac muscle was used as the source of cells in explant cultures, and with both fixation procedures. Similar results were also obtained when cells cultured from control birds were examined. These findings are summarised in Table 1. Only very faint diffuse staining was seen when preimmune serum from the same rabbit was used, or when the fluorescein-labelled goat IgG alone was used.

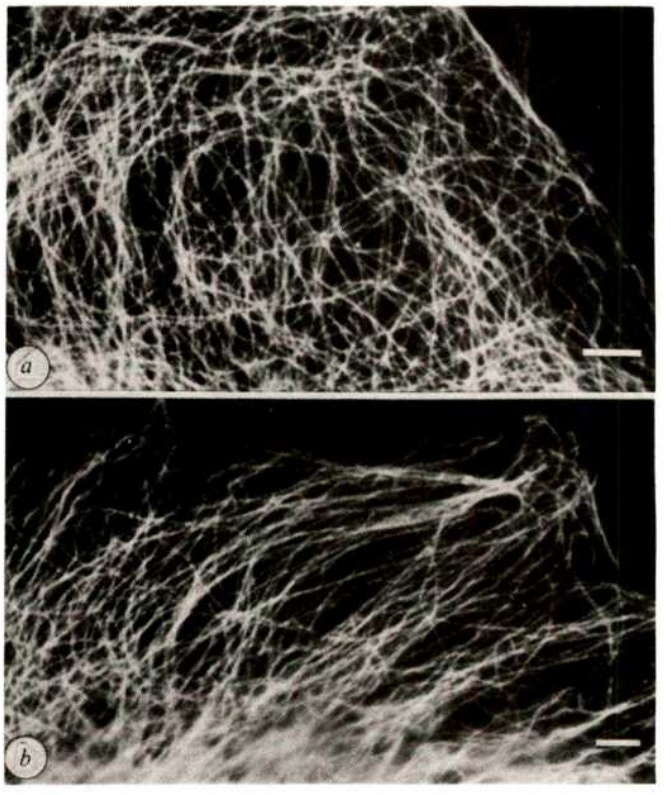


Fig. 2 Cells from dystrophic (*a*) and normal (*b*) explants of skeletal (leg) muscle from 15-d-old chickens stained with antiserum to tubulin. No differences are apparent in these higher magnification views of the microtubular network. Scale bar, 5 μm.

In a complementary study, we also examined microtubule organisation in skin fibroblasts of persons with DMD. In cultured fibroblasts from three individuals with DMD and two controls, no differences were seen between normal and dystrophic cells, and in all cases an extensive network of microtubules was visible in more than 99% of the cells. Figure 3 shows the clearly evident microtubular network present in cultured dystrophic (Fig. 3*a*) and normal(Fig. 3*b*) human skin fibroblasts. Again, similar results were obtained using either fixation method.

We were unable to detect significant differences in the organisation of the cytoplasmic network of microtubules between cells derived from dystrophic chicken muscle and control cells. Likewise, we were unable to detect differences in microtubule organisation between cultured fibroblasts from

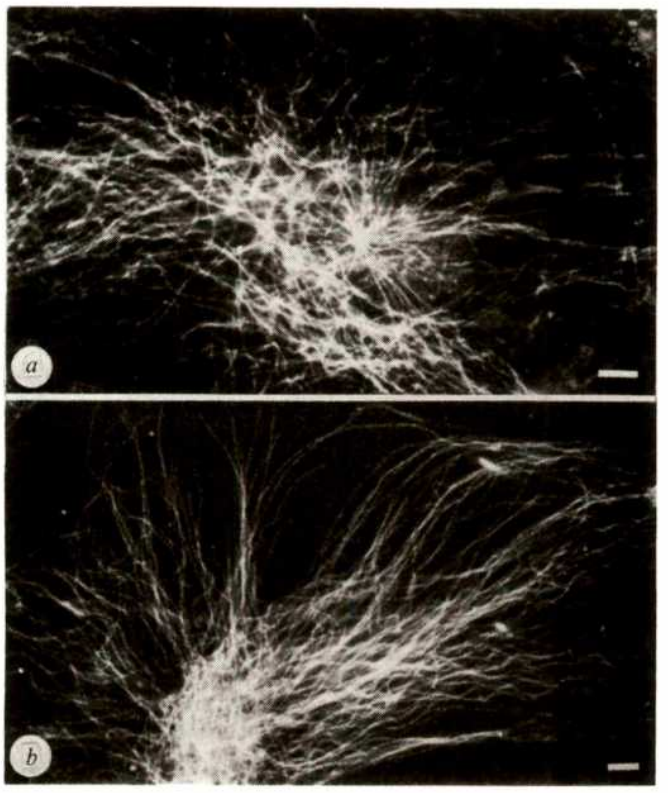


Fig. 3 Human skin fibroblasts from a person with DMD (*a*) and a normal control (*b*) stained with antiserum to tubulin. A similar cytoplasmic network of microtubles is present in both cells. Scale bar, 5 μm.

persons with DMD and control subjects. These results contrast with those reported by Shay and Fuseler[5]. The methods we used for explant cultures were identical to those described in their report[5]. Like Shay and Fuseler, we observed that both normal and dystrophic interphase cells are flat and well-spread on the substrate, and that the distribution of actin in these two cell types is similar (B.H.B., unpublished results). Therefore, we do not understand the discrepancy between their results and ours with respect to the distribution of microtubules in dystrophic cells. The dystrophic chickens from which we prepared our cells were of the Connecticut line, whereas Shay and Fuseler used cells from line 413 birds[5]. Although the difference in genetic background could contribute to the observed differences, it seems unlikely to represent a major factor because both chicken lines are derived from the original 301 dystrophic line, possess the same dystrophic gene (*am*) and are characterised by similar manifestations of the disease[8]. Differences between the antiserum used by Shay and Fuseler and our antiserum may also account in part for the discrepancy in results. However, we believe a more likely explanation lies in the different fixation procedures used and that our methods enhanced the visualisation of microtubules by reducing background staining.

In conclusion, we have been unable to detect major differences in the organisation of microtubules between cells from dystrophic individuals and their corresponding control cells. On the basis of these findings, therefore, we are unable to support any correlation between the expression of the dystrophic genotype and a reduction in the number of microtubules in the interphase cells of affected individuals.

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Table 1 Cytoplasmic microtubules in chicken cells

Cell type	Age of animal	% Positive* cells
E–NS	12d (e)	99.5
E–NC	12d (e)	99.0
E–DS	12d (e)	99.5
E–DC	12d (e)	99.5
E–NS	1d (h)	98.5
E–NC	1d (h)	99.0
E–DS	1d (h)	98.5
E–DC	1d (h)	98.5
E–NS	15d (h)	100.0
E–NC	15d (h)	99.0
E–DS	15d (h)	98.5
E–DC	15d (h)	98.5
T–NS	12d (e)	100.0
T–DS	12d (e)	100.0
T–NS	1d (h)	100.0
T–DS	1d (h)	100.0

E, cells from muscle explant cultures; T, cells from trypsinised muscle tissue; NS, normal skeletal muscle; NC, normal cardiac muscle; DS, dystrophic skeletal muscle; DC, dystrophic cardiac muscle; e, embryonic; h, hatchling.

* In each case 200 cells were counted and cells were considered positive only if numerous microtubules of extended length were visible in the cytoplasm.

Different receptors for distribution of peanut and ricin agglutinins between inner and outer segments of rod cells

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Lectins can be used as probes for cell-surface oligosaccharides[1–4]. These proteins display high specificities for certain haptene sugars, although the details of the sugar linkages and the three-dimensional array of the oligosaccharide may all be involved in determining the affinity of a lectin for its receptor. We have now shown that peanut and ricin agglutinins bind differentially to the surfaces of rod inner and outer segments.

Receptors for jack bean lectin (concanavalin A, Con A) are present on the surfaces of rod outer segments (ROS)[5–7]. Con A has a specificity for complex mannose-containing oligosaccharides with internal and terminal *N*-acetylglucosamine residues[8–11] as well as simpler structures containing mannobiosyl units[12]. The high affinity of Con A for rhodopsin[13] is consistent with recent observations showing that its oligosaccharide has the structure GlcNAcβ1→2Manα1→3(Manα1→6)Manβ1→4GlcNAcβ1→4GlcNAc→Asn (refs 14, 15). Because rhodopsin is probably present in the plasma membrane of the ROS as well

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as the bimembranous disks found in its interior[16,17], it may represent the majority of the Con A receptors. A minor ROS protein, with a molecular weight of 291,000 (refs 18, 19), has also been shown to bind Con A[20], and probably does not play a significant part in binding Con A to the ROS surface.

Some rhodopsin molecules may carry terminal D-galactose residues[21], so we also investigated the galactose-specific agglutinins from *Ricinus communis* (RCA_I) and *Arachis hypogaea* (PNA). These lectins have been used to suggest the existence of terminal galactose units in, for example, normal 3T3, transformed SV3T3, transformed 3T12 cells and embryonal carcinoma cells[22,23].

PNA (L'Industrie Biologique Française) and RCA_I (Sigma and PL-Biochemicals) caused agglutination in suspensions of washed ROS at concentrations of 11 and 25 $\mu g\ ml^{-1}$, respectively. The effect was inhibited by D-galactose at a concentration of 4–40 mM for PNA and 0.3–3 mM for RCA_I. Lactose and α-D-(but not α-L-) fucose inhibited agglutination by RCA_I. Other sugars tested, and found to be ineffective, were α-methyl mannopyranoside, D-glucose, *N*-acetyl glucosamine, chitobiose and *N*-acetyl galactosamine. For comparison, Con A agglutinated ROS at 0.1–0.5 $\mu g\ ml^{-1}$, and was inhibited by α-methyl-mannopyranoside and glucose (see ref. 5).

Fresh ROS from light- or dark-adapted frogs were incubated with fluorescein isothiocyanate (FITC)-labelled PNA or RCA_I and compared, using a Zeiss epifluorescence microscope, with ROS incubated with FITC–Con A. In such preparations a variable proportion of the ROS had part of the inner segment attached. Figure 1 is a typical fluorescence photograph of one of these organelles after incubation with FITC–Con A. Bright surface fluorescence is visible on both the outer and inner segment, showing that receptors for Con A are present at both locations. A brightly fluorescent band delineates the junction between inner and outer segment, perhaps attributable to penetration of the lectin into the few open disks known to occur at that point[24]. The surface fluorescence was virtually abolished if incubation was carried out in the presence of 100 mM α-methyl mannopyranoside.

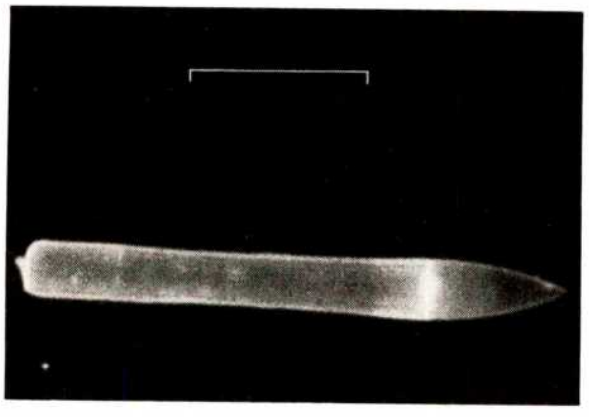


Fig. 1 Fluorescence photograph of an isolated frog (*Rana pipiens*) rod outer segment with part of inner segment attached. FITC–Con A, 100 $\mu g\ ml^{-1}$ in frog Ringer, 12 min incubation at room temperature. Scale bar, 20 μm.

In contrast, Fig. 2 is a similar preparation incubated with FITC–PNA. Although the fluorescence intensity is lower than that for Con A, and hence more difficult to photograph, it can be seen that the bright line of the outer segment surface label stops abruptly at the inner segment, and that there is a dividing line of similar intensity between the two sectors. The diffuse, low-level fluorescence occupying part of the inner segment is autofluorescence which develops in the region of tightly packed mitochondria. It is straw yellow in colour, in contrast to the characteristic green emission of the FITC label. RCA_I gave an

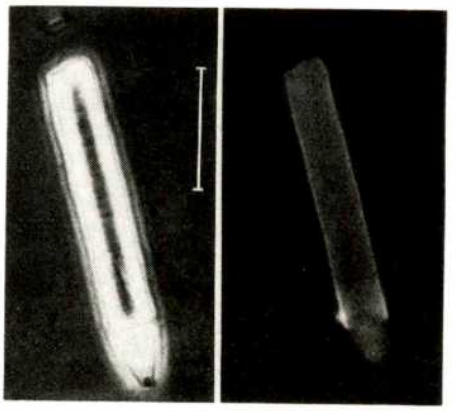


Fig. 2 Preferential binding of FITC–PNA to outer segment plasma membrane. Left hand side, normal phase illumination; right hand side, fluorescence. Both photographs were taken on Kodak Tri-X film pushed to an effective ASA rating of 1,600 with Diafine. Virtually no fluorescence is visible on the plasma membrane of the inner segment; diffuse inner segment fluorescence is autofluorescence from the mitochondria. FITC–PNA, 200 $\mu g\ ml^{-1}$, 12 min at room temperature. Scale bar, 20 μm.

identical pattern of labelling. The binding of both lectins was abolished in the presence of 100 mM D-galactose. After prolonged incubation with high lectin concentrations, a very faint surface label was occasionally seen on the inner segment, but in all cases this was many times weaker than that of the outer segment.

Although PNA has its highest affinity for the disaccharide D-galβ1→3GalNAc (ref. 25), characteristic of many blood glycoproteins, it shares with RCA_I a specificity for the simple terminal sugar D-galactose. The difference between inner and outer segment membranes in their ability to bind RCA_I and PNA suggests that oligosaccharides present on the inner segment plasma membrane lack D-galactose in an accessible, terminal position. Rapid translational and rotational freedom for rhodopsin has been demonstrated in rod disk membranes, indicating that they have an unusually low viscosity[26]. A similar fluidity in the plasma membrane enveloping the whole rod cell would permit RCA_I and PNA receptors to diffuse through the inner–outer segment junction. Their failure to do this may indicate the existence of a barrier at this point, or the plasma membrane may be more rigid than the disk membranes.

The synthetic machinery of the photoreceptor is found in the inner segment, but the finding that oligosaccharides binding RCA_I and PNA are restricted to in the outer segment suggests that only in this region of the cell have they been incorporated into the membrane, or their terminal sugars modified. The receptors themselves may be glycolipids or galactosylated rhodopsin molecules. As noted above, rhodopsin molecules constitute many of the Con A receptors in the outer segment plasma membrane. However, the binding observed on the inner segment (down to the synaptic pedicle in papain-dissociated material) may not involve rhodopsin because Con A binds to sugar sequences common to many glycoproteins[27–29].

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Enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia

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Recent biochemical and immunohistochemical studies have shown that the opioid peptides, enkephalins, occur in nerve terminals and cell bodies in mammalian sympathetic ganglia[1–3]. Opiates and enkephalins are thought to inhibit synaptic transmission in the peripheral nervous tissues as well as in the central nervous system[4–12]. The mechanisms of the opiate actions, however, are not entirely clear; both pre- and postsynaptic sites of action have been proposed[7–9,11,12]. As acetylcholine is known to be the major neurotransmitter in the autonomic ganglia and as the mechanism of synaptic transmission is well clarified[13], analysis of the peptide action could be more easily but equally usefully carried out in the peripheral synapses than in central synapses. We now report that enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia.

The experiments were carried out on the coeliac-superior mesenteric and inferior mesenteric ganglia isolated from male guinea pigs weighing 200–400 g. The animals were stunned and bled. The ganglia together with associated nerves were dissected and incubated with 0.02% collagenase for about 30 min to facilitate microelectrode impalement. The preparations were pinned out in a bath (0.5 ml capacity) made of silicon resin and continuously perfused at a flow rate of 2–4 ml min^{-1} with modified Tyrode solution of the following composition (mM): NaCl, 138.6; KCl, 3.4; $CaCl_2$, 1.3; $MgCl_2$, 1.2; $NaHCO_3$, 21; NaH_2PO_4, 0.6; glucose, 10, equilibrated with 95% O_2 and 5% CO_2. The solution was kept at a temperature of 35–37°C. In some experiments solution containing low calcium (0.7 mM) and high magnesium (2 mM) was used to produce a partial block of synaptic transmission in the ganglia. For stimulating the preganglionic nerve trunks, the greater and/or lesser splanchnic nerves of the coeliac-superior mesenteric ganglion or the lumbar splanchnic and/or intermesenteric nerves of the inferior mesenteric ganglion were drawn into suction electrodes (for the terminology of the nerves see ref. 14). Intracellular recordings were made with glass microelectrodes filled with 2 M potassium acetate, with resistances of 40–80 MΩ. Signals were led to a preamplifier and the output was displayed on an oscilloscope and a pen recorder. A conventional bridge circuit incorporated in the preamplifier was used for recording and passing current pulses through a single microelectrode. Hyperpolarising current was usually injected into the cells to prevent spike generation in response to preganglionic stimulation. In most cases, the size of the response was measured after averaging with a TMC-300 signal averager (Kawasaki). Results were obtained from 53 cells with resting potentials of −45 to −80 mV and action potentials of 70–105 mV.

Met-enkephalin and Leu-enkephalin in concentrations of 0.5–20 μM depressed the excitatory postsynaptic potentials (e.p.s.ps) evoked by preganglionic stimulation in the coeliac-superior mesenteric and inferior mesenteric ganglia. The effect of the peptides was similar in both ganglia. [D-Ala^2]-methionine-enkephalinamide (DAEA), a metabolically stable Met-enkephalin analogue[15], was also effective and had a potency over 30 times higher than that of Met-enkephalin (Fig. 1*A*). The response to DAEA showed neither desensitisation during a 3-min exposure period nor tachyphylaxis with repeated applications when the interval between each application was more than 10 min. The inhibitory effect of DAEA was more marked in low-calcium and high-magnesium solution than in the control solution. DAEA in concentrations of 0.3–2 μM reduced

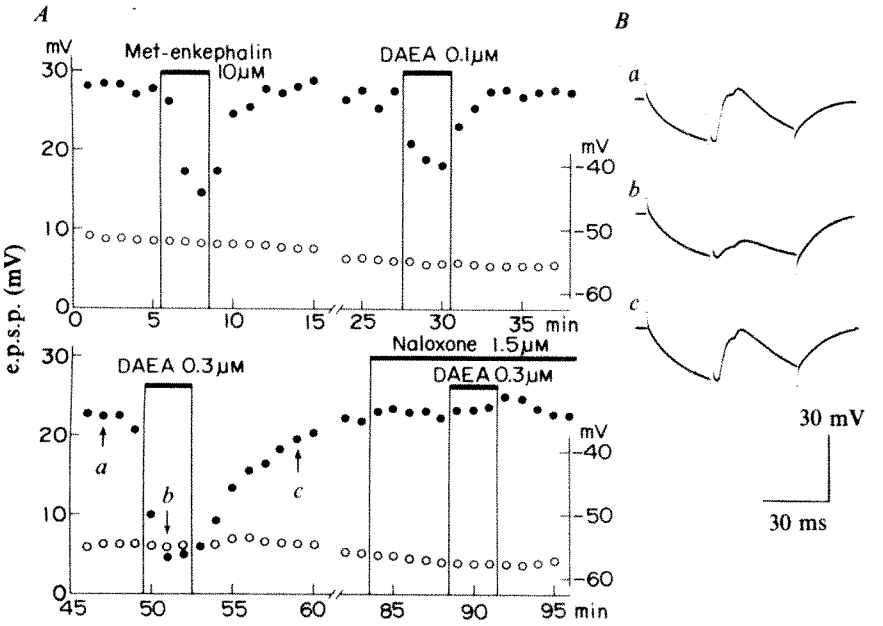


Fig. 1 Effects of Met-enkephalin and DAEA on e.p.s.ps, resting potential and membrane resistance of a postganglionic cell in the inferior mesenteric ganglion, and antagonism by naloxone. The preparation was perfused with solution containing low calcium (0.7 mM) and high magnesium (2 mM) concentrations. Hyperpolarising current pulses of 70 ms duration and 0.5 nA intensity were passed into the cell to prevent spike generation following the e.p.s.p. and also to estimate the membrane resistance. The lumbar splanchnic nerve was stimulated at 1-min intervals for 16 s at 2.5 Hz. *A*, The amplitude of the e.p.s.ps (●) was determined at 1-min intervals after averaging 32 responses. The resting potential (○) was measured from the continuous record of the pen recorder. Ordinate: left, amplitude of the averaged e.p.s.ps; right, resting potential. Each drug was perfused into the bath during the period indicated in the graph. *B*, *a–c*, Sample records of the averaged e.p.s.ps and the potential changes caused by current injection. Records were obtained at arrows *a–c* of *A*.

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the amplitude of e.p.s.ps by 52.3±4.4% (mean±s.e.m., n = 14), without significantly affecting either the resting potential (Fig. 1*A*) or the membrane resistance, which was estimated by passing constant hyperpolarising current pulses (Fig. 1*B*) or by observing the current–voltage relationship. The inhibitory effect of DAEA (0.3–1.0 μM) was abolished by the opiate antagonist naloxone (1–3 μM), suggesting that the peptide inhibition results from a specific action on opiate receptors (Fig. 1*A*). Naloxone alone did not produce any consistent change in the size of e.p.s.ps.

number of quanta released by each stimulus. DAEA (1–2 μM) increased the number of failures (see Fig. 2*B*, *C*) and reduced the mean quantum content of the e.p.s.ps to 40–60% of the control (n = 3). However, the peptide did not cause any significant change in the mean amplitude of the quantal unit (Fig. 2*C*). The results support the presynaptic nature of enkephalin-induced inhibition.

We also observed that noradrenaline depressed excitatory synaptic transmission in the coeliac-superior mesenteric ganglion, confirming the previous results of Christ and Nishi[17].

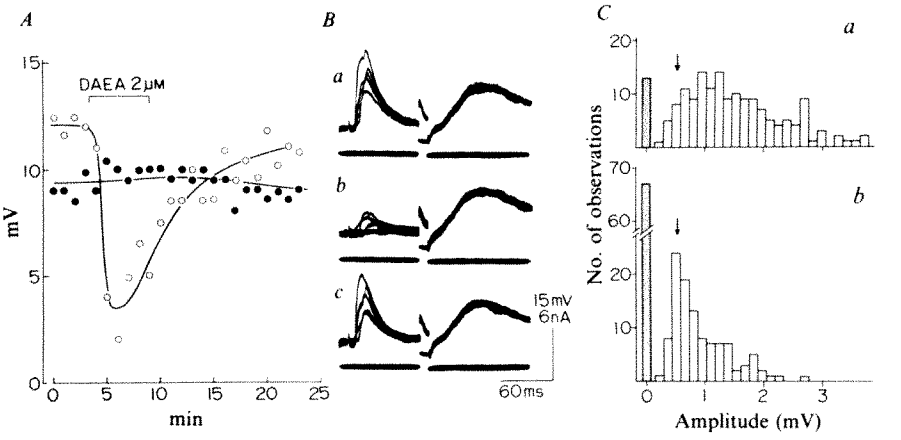


Fig. 2 Effect of DAEA on the e.p.s.p. and acetylcholine-induced depolarisation of the postganglionic cell in the coeliac-superior mesenteric ganglion, and on amplitude histograms of e.p.s.ps. In *A* and *B* the greater splanchnic nerve was stimulated at 1-min intervals by 16 pulses at 0.5 Hz. Acetylcholine was iontophoretically applied to the cell about 80 ms after each nerve stimulus. *A*, The amplitudes of the e.p.s.ps (○) and the acetylcholine-induced depolarisation (●) were determined at 1-min intervals after averaging 16 responses. The peptide was perfused into the bath during the period indicated. *B*, *a*–*c*, Upper trace shows the e.p.s.ps (left) and acetylcholine-induced depolarisation (right), and lower trace shows the current passed into the acetylcholine-containing electrode. *a*, Control; *b*, 2–3 min after addition of DAEA (2 μM). *c*, 7–8 min after washing out DAEA. Hyperpolarising current (0.1 nA) was continuously passed into the cell to prevent spike generation. *A* and *B* were obtained from the same experiment. *C*, Amplitude histograms of e.p.s.ps evoked by stimulation of the greater splanchnic nerve at 2 Hz. *a*, Control; *b*, in DAEA (1 μM). The quantum content, m, was estimated from the numbers of stimuli, n, and failures (stippled bar). The estimated unit sizes of the e.p.s.ps are shown by arrows. For *a* and *b*, respectively, n was 167 and 176, and m was 2.55 and 0.97.

To test the possibility that enkephalins act directly on the postsynaptic membrane and reduce the sensitivity to acetylcholine, the effect of DAEA on acetylcholine response was examined. An intracellular recording electrode was inserted into superficial cells and acetylcholine was applied iontophoretically from another microelectrode placed on the surface of the impaled cells. Short pulse application of acetylcholine produced a depolarisation of the postganglionic cells. The size and time to peak of the depolarisation varied greatly depending on the position of the drug-containing electrode and the applied current. As shown in Fig. 2, the amplitude and time course of the acetylcholine-induced depolarisation were not affected by DAEA, whereas the e.p.s.ps recorded simultaneously were markedly reduced in size. Both the e.p.s.ps and the acetylcholine-induced depolarisation were almost completely abolished by dihydro-β-erythroidine (5 μM). These observations suggest that enkephalins inhibit the release of acetylcholine from the preganglionic terminals but do not reduce the sensitivity of nicotinic receptors on the postganglionic cells.

The undecapeptide, substance P, which also has been demonstrated in the sympathetic ganglia[16], caused a depolarisation of up to 20 mV in certain postganglionic cells. The substance P-induced depolarisation was not depressed by tetrodotoxin (1 μM), which completely blocked synaptic transmission in these ganglia. This suggests that substance P acts directly on the postsynaptic membrane. As observed with the acetylcholine response, the direct depolarising action of substance P(0.4–1.0 μM) was not affected by DAEA (1–2 μM), suggesting that enkephalins have no effect on the chemosensitivity of the postsynaptic membrane.

To confirm the site of enkephalin action, amplitude histograms of e.p.s.ps evoked by preganglionic stimulation at 1–2 Hz in the low calcium (0.7 mM) and high magnesium (2 mM) solution were examined before and after addition of DAEA. Assuming a Poisson distribution of the e.p.s.ps and counting the number of failures in a series of trials, we estimated the mean

However, the possibility that the inhibitory effect of enkephalins might be mediated by the release of a catecholamine was excluded by the observation that the inhibition caused by DAEA (1 μM) was not affected by phentolamine (3 μM), which effectively antagonised the inhibitory effect of noradrenaline (5 μM).

Our results suggest the possible involvement of enkephalins in presynaptic inhibition of cholinergic transmission in mesenteric ganglia. This is particularly relevant to morphological evidence that some of the preganglionic fibres form axo-axonic synapses in the cat inferior mesenteric ganglion[18]. Furthermore, the mode of enkephalin action shown in this study is consistent with recent evidence that enkephalins depress primary afferent transmission in the spinal cord and brain stem, and inhibit the release of substance P, a putative neurotransmitter of the primary afferent fibres[7,8,19].

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ADP is a potent inhibitor of human platelet plasma membrane adenylate cyclase

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The potent inducer of platelet aggregation, ADP, inhibits both basal and stimulated cyclic AMP production in intact platelets[1,2]. However, several attempts have failed to detect an effect of the nucleotide on adenylate cyclase activity in broken cell preparations[2,3]. This paradox has led to suggestions that ADP may act on unidentified sites before the adenylate cyclase step, such as a nucleoside diphosphate kinase[4] and a Ca^{2+}-transport system[5,6]. It is difficult to demonstrate the effect of physiologically effective concentrations (0.1–5 μM) of ADP on a crude membrane preparation of adenylate cyclase because of the presence of nucleoside phosphohydrolase activities which may degrade either the substrate ATP or the putative effector ADP to products (such as ADP, AMP and adenosine) displaying diverse effects. A substrate regenerating system which converts ADP to ATP obviously provides no solution. The contamination by adenine nucleotides and catecholamines in widely used crude lysate preparations aggravates the problem[7–9]. Using a purified human platelet plasma membrane preparation, and the non-hydrolysable ATP analogue APP(NH)P as substrate, we have now demonstrated direct inhibition of adenylate cyclase by ADP in the physiologically effective concentration range. The inhibitory effect of ADP is immediate in onset and is equal to that produced by adrenaline; it is shared by ADP-βS (and 2′-deoxy ADP to some extent) but not by AP(CH₂)P or other adenine nucleotides. The K_i for ADP is ~0.3 μM and maximal inhibition is observed at 2 μM.

In these studies membranes were prepared essentially as described by Barber and Jamieson[10]. The procedure involves lysis of glycerol-loaded freshly drawn washed human platelets, followed by sucrose gradient centrifugation of the particulate fraction and collection of the plasma membrane-enriched region at 60,000 g. Membranes were washed before use with 0.4 M NaCl in 20 mM Tris, *p*H 7.4, centrifuged at 10,000g, 10 min, 4 °C and resuspended in 10 mM Tris *p*H 7.4 containing 0.1% bovine serum albumin and 0.5 mM EGTA. Assays were carried out at 24°C, generally for 6 min in an incubation volume of 100 μl containing 2–4 μg membrane protein, 0.04% bovine serum albumin, 0.2 mM EGTA, 30 mM Tris *p*H 7.4, 2 mM $MgCl_2$, 40 μM APP(NH)P (Böhringer, lot 31), 1 μCi [α-^{32}P]APP(NH)P, 50 μM cyclic AMP with other additions as indicated. ^{32}P-labelled cyclic AMP was isolated as described elsewhere[11]. Triplicate or quadriplicate determinations of activity were normally performed in a block design and standard deviations of means were generally <5%. When assessed by TLC on PEI-cellulose[12], substrate concentration remained constant (>95%) during the routine assay period.

Table 1 Effect of ADP and related compounds on platelet adenylate cyclase activity

Additions (2.5 μM)	Cyclic AMP (pmol per min per mg protein)	% of control
—	83.8 (5.5)	100
Adenosine	81.2 (1.6)	97
2′-Deoxy AMP	82.4 (1.76)	98
AP(CH₂)P	82.0 (1.09)	98
AMP	79.7 (2.56)	95
2′-Deoxyadenosine	77.0 (3.8)	92
2′-Deoxy ADP	75.6 (3.36)	90
ADP	64.6 (3.15)	77
ADP-βS	63.6 (0.63)	76

Adenylate cyclase activity was determined as described in Fig. 1 legend with both 1 μM PGE_1 and 1 μM GTP in the presence of either 2.5 μM ADP or the related compounds indicated. Values presented are the means ±s.d. of quadruplicate determinations. Activities are also expressed as a percentage of that observed in the absence of the ADP analogues.

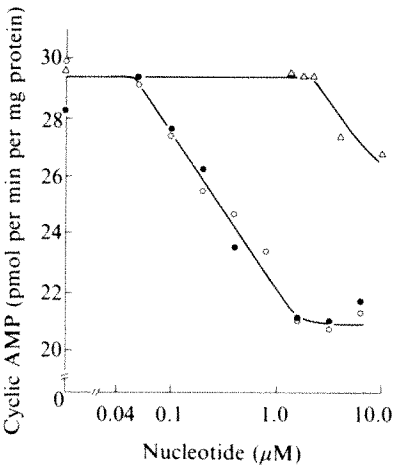


Fig. 1. Effect of ADP, ADP-βS and AP(CH₂)P on human platelet plasma membrane adenylate cyclase activity. Enzyme activity was measured in the presence of 1 μM PGE_1 and 1 μM GTP using 40 μM APP(NH)P, 2 mM $MgCl_2$ and 0.2 mM EGTA in the presence of the indicated concentrations of ADP (●), ADP-βS(○) and AP(CH₂)P (△).

The effects of ADP, ADP-βS (Böhringer) and AP(CH₂)P (ICN) on platelet adenylate cyclase activity are compared in Fig. 1. A potent inhibition of activity (K_i: 0.3 μM) which is maximal at 2 μM is displayed by both ADP and ADP-βS. AP(CH₂)P is only weakly effective. The very similar behaviour of ADP and ADP-βS demonstrates that no phosphorylation of the nucleotide is required for its effect since the thiol analogue cannot be phosphorylated. The ineffectiveness of AP(CH₂)P correlates well with intact platelet studies which showed that this analogue acted only as an antagonist of ADP and was without effect on platelet aggregation[13,14] at physiological *p*H. Evidence has been presented[13] which suggests that part of the ineffectiveness of this analogue resided in the pK_a value of the secondary phosphoryl hydroxyl group, which is 8.0 for the analogue compared with 7.0 for the natural nucleotide[15]. Additionally, altered stereochemistry of the phosphate chain due to the substitution of a (hydrophobic) methylene group has been invoked to account for the lack of effect of the compound[14].

The K_i value of ~0.3 μM for ADP (and ADP-βS) inhibition of adenylate cyclase in the plasma membrane preparation in the present study compares well with the K_i value (0.5 μM) for the inhibition by ADP of cyclic AMP production in intact platelets[2,6]. However, the amount of inhibition (~25%) elicited by ADP in the present study compares with ~90% inhibition of cyclic AMP production in intact platelets. No immediate explanation for this discrepancy is available, but it may suggest either the unmasking of additional pools of adenylate cyclase or some deregulation of the activity on the disruption of cells and preparation of membranes. There was a similar degree of inhibition of fat cell adenylate cyclase by adenosine analogues which was observed as a dramatic reduction in cellular cyclic AMP levels[16]. Sabol and Nirenberg[17] reported similar levels of inhibition of neuroblastoma–glioma hybrid adenylate cyclase by α-adrenergic agents and other opiates.

The effects of a more extensive range of ADP concentrations were also examined. After the sensitive phase of ADP inhibition

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which is complete at 2 μM (compare Fig. 1), a plateau in activity follows. Further inhibition is evoked by concentrations >40 μM(K_i~200 μM), which presumably represents competition of ADP for substrate at the catalytic site (results not shown).

PGE_1-stimulated activity with and without GTP is shown as a function of time in the presence and absence of 2 μM ADP in Fig. 2. Activities are linear for 10 min in the absence of ADP. Inhibition by ADP is immediate in onset and is maintained throughout the usual assay period (6 min). After 6 min there is some diminution in the amount of inhibition caused by ADP, which may reflect ADP metabolism.

Degradation of ^{14}C-ADP (2 μM) during the routine assay period (6 min) was assessed by TLC on PEI-cellulose[12] of an aliquot of reaction mixture to which [α-^{32}P]APP(NH)P had not been added. At least 85% of the radioactivity applied was recovered in the ADP region in two determinations (results not shown). The low level of degradation of ADP by a plasma membrane preparation, presumably containing nucleotide phosphohydrolases, may reflect protection of the diphosphate by the adenylate cyclase substrate, APP(NH)P.

In Table 1 the effect of ADP on platelet adenylate cyclase is compared with that of ADP analogues and related compounds. ADP and ADP-βS yield equal inhibition, AP(CH_2)P is ineffective and 2'-dADP gives intermediate results. The relative ineffectiveness of AMP, dAMP, adenosine and 2'-deoxyadenosine underline the fact that ADP and ADP-βS act on a receptor which is specific for ADP as first suggested by Born[18]. The weak inhibition by 2'-deoxyadenosine may be viewed as an interaction with inhibitory adenosine sites described previously[9,19].

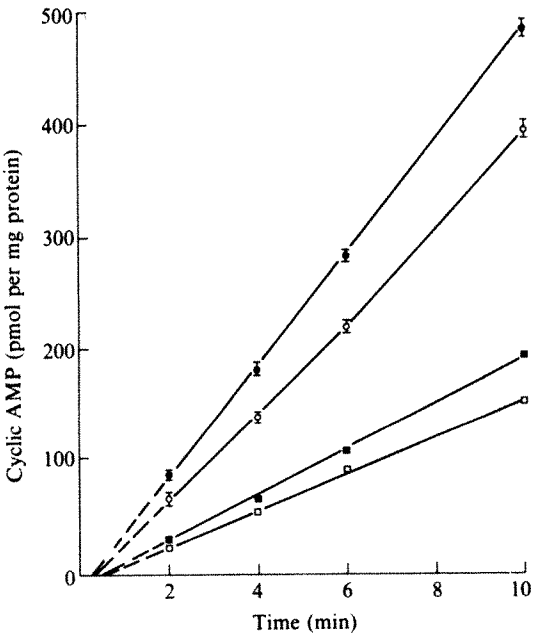


Fig. 2 Time course of the effect of ADP on adenylate cyclase activity of platelet plasma membranes. Cyclic AMP accumulation was measured at the times indicated in the conditions described in Fig. 1 in the presence of 1 μM PGE_1 alone (■, □) or in combination with 1 μM GTP (●, ○) without (●, ■) or with (○, □) 2 μM ADP. Standard deviations of triplicate determinations are shown for the upper two lines.

Table 2 shows the inhibition by ADP of various activity states of platelet adenylate cyclase activity. Clearly the greatest absolute amount of inhibition is obtained with maximally stimulated activities. It is not so clear whether the ADP inhibition is preferentially directed towards a particular state. A notable feature is that the amount of inhibition evoked by ADP is equivalent to that produced by adrenaline in this experiment.

Table 2 Effect of ADP on variously stimulated platelet adenylate cyclase

	Cyclic AMP (pmol per min per mg protein)		
Additions	−ADP	+ADP(2 μM)	% activity in absence of ADP
GTP (1 μM)	4.79 (0.29)	3.5 (0.15)	73
GTP + PGE_1 (1 μM)	72.9 (4.4)	56.9 (1.02)	78
GTP + EPI (10 μM)	3.74 (0.46)	3.44 (0.33)	92
GTP + EPI + PGE_1	51.0 (4.1)	44.8 (1.17)	88

Adenylate cyclase activity was determined as described in Fig. 1 in the presence or absence of 2.5 μM ADP with 1 μM GTP, 1 μM PGE_1 and 10 μM (−)L-adrenaline as indicated. Values presented are the mean ± s.d. of quadruplicate determinations. Activities are also expressed as a percentage of that observed in the absence of ADP.

Jakobs *et al.*[8] reported a similar degree of inhibition of platelet adenylate cyclase by adrenaline. Interestingly, the combination of ADP and adrenaline yields a total of 40% inhibition of activity relative to control values (Table 2, 72.9 to 44.8) but the relative amount of inhibition induced by ADP is diminished in the presence of adrenaline and vice versa. These findings suggest that the two agents converge on a common pool of catalytic activity.

The present report has described a direct inhibition of platelet plasma membrane adenylate cyclase activity by ADP in the same concentration range as that which is effective in both reducing cyclic AMP production in intact platelets and promoting platelet aggregation. The detection of this effect of ADP on a membrane adenylate cyclase also opens up the possibility of detecting such effects on the enzyme in other systems. Primary candidates are those physiological systems thought to be mediated by 'purinergic receptors' as described by Burnstock[20] and those systems in which adenylate cyclase shows different behaviour when APP(NH)P rather than ATP is used as substrate (compare ref. 21).

ADP inhibits as effectively as adrenaline in this system. A combination of the two agents evokes greater inhibition than that elicited by either agent alone. Precisely how these effectors interact in the regulation of platelet adenylate cyclase remains to be established. However, it is possible that this interaction may serve as a model of the means whereby neurotransmitters and adenine nucleotides, which are commonly secreted together in synapses, participate in the 'neuromodulation' of physiological events[20].

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Concentration dependence of currents through single sodium-selective pores in frog skin

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Many transport processes in biological membranes show a saturation of transport rate when the concentration of the transported ions or molecules is sufficiently increased. Saturation will result when at high concentrations a translocation step which does not directly depend on bulk concentration becomes rate limiting, and it is expected for all multi-step translocation mechanisms. However, a possible alternative explanation for saturation is a regulatory process which decreases the number of translocating channels in response to an increase in bulk concentration of the transported species. In the frog epidermis, Na^+ uptake from the extracellular space into the epithelial cytosol occurs through Na^+-selective, passively conducting channels located in the apical membrane of the cells directly below the cornified outer cell layer. Saturation of transport occurs with increasing outer Na^+ activity ($[Na]_o$): the net Na^+ current[1,2], membrane conductance[2,3] and unidirectional Na^+ influx[4] increase only subproportionally with $[Na]_o$. The apical Na^+ current can transiently be larger than the saturation value, suggesting that a fixed upper single channel transport rate is not responsible for the saturation, and it has, therefore been suggested that in this case saturation occurs through a $[Na]_o$-dependent decrease in channel density[2]. The Na^+-translocation process can be investigated by a statistical evaluation of spontaneous transport fluctuations. The reversible transport blocker amiloride can be used to induce random fluctuations of the Na^+ current at the apical membrane. The power density spectrum of these fluctuations is of the lorentzian type and permits an estimation of the Na^+ current which passes through individual translocators in the time intervals between blocks[5]. We have recorded the current fluctuations at different $[Na]_o$ values and different amiloride concentrations in the $[Na]_o$ range in which saturation of total Na^+ current is observed. We report here that the density of the conducting pores decreases with increasing $[Na]_o$.

The experimental technique was similar to that described previously[5]. We used abdominal skins of *Rana esculenta* at room temperature in a Lucite chamber with 3 cm^2 exposed to the bathing solutions. The tissue was held by silicon rings (Silgel 604, Wacker Chemie) to avoid damage to the edges. The inner solution was K^+-sulphate Ringer, which would be expected to depolarise the K^+-selective inward-facing membranes of the epithelium and produce a four- to five-fold increase in their conductance. At high inner K^+ concentrations the epithelium (judged by its current–voltage curve) behaves approximately like a one-membrane preparation[2]. Transepithelial resistance and potential are then largely determined by the Na^+-selective, outward-facing membrane. This situation was further improved by using amiloride to increase the resistance of this membrane. The outer solution was sulphate Ringer in which the Na^+ concentration was varied by substitution with K^+. An activity coefficient of 0.55 was used to compute Na^+ activities[2,6]. We used $[Na]_o$ values of 6–60 mM and amiloride concentrations, $[A]_o$, of 1–16 μM for fluctuation analysis. I_{Na}, the transcellular current component which could be blocked by amiloride, was determined as described elsewhere[5]. The transepithelial voltage (V) was clamped to zero with a voltage-clamp circuit designed for low-noise applications[7]. The fluctuating component of the feedback current was amplified and sampled as described previously[5,8].

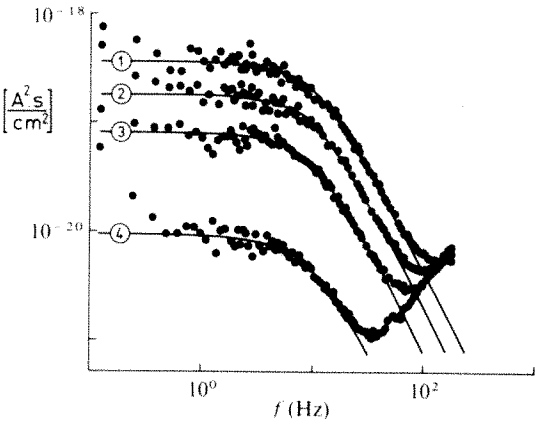


Fig. 1 Power density spectra obtained at constant amiloride concentration (4 μM) and four values of $[Na]_o$, as indicated. Power densities are normalised to 1 cm^2. It can be seen that a 10-fold variation in $[Na]_o$ causes significantly less than a 100-fold variation in S_0^A (plateaus of Lorentz curves), and a small change of the corner frequency f_c^A. In the high frequency range the excess noise becomes smaller than the amplifier noise, which increases because of the decrease in the membrane impedance at high frequencies. Respective values for f_c^A (Hz) and $[Na]_o$ (mM) are: ① 10.5, 60; ②, 10.4, 30; ③, 9.5, 18; ④, 9.3, 6.

In agreement with previous results, the amiloride-induced component of the spectrum of the Na^+ current was found to have a corner frequency, f_c^A, which increased with the amiloride concentration, whereas the plateau values S_o^A, of the corresponding lorentzian curve decreased. f_c^A increased linearly with $[A]_o$ values between 3 and 12 μM, and so the amiloride–receptor reaction could be described by first order kinetics using the reaction rate ($1/\tau$)

$$2\pi f_c = \frac{1}{\tau} = k_1'(A)_o + k_2 \qquad (1)$$

where k_1' represents the apparent association rate constant, k_2 the dissociation rate constant and τ the relaxation time constant[5]. Corner frequencies smaller than predicted by this straight-line relationship were often found with $[A]_o$ values of 1, 2 and 16 μM. At these amiloride concentrations the power density spectra usually deviated markedly from the lorentzian, probably because of additive spectral components— at low $[A_o]$ the amiloride spectrum is too close to the spectrum of the spontaneous fluctuations to be easily evaluated, and at high $[A]_o$ it is too close to the background spectrum obtained from the epithelium in Na^+-free solutions. Therefore, we have restricted our analysis to amiloride concentrations between 3 and 12 μM. At constant $[A]_o$, f_c^A increased by about 1 Hz in response to a 10-fold increase in $[Na]_o$ (see Fig. 1). With $[Na]_o = 60$ mM, $2\pi f_c^A$ was calculated to be $62.1 \pm 1.7\ s^{-1}$ at $[A]_o = 4$ μM ($n = 10$), and $115.9 \pm 3.2\ s^{-1}$ at $[A]_o = 8$ μM ($n = 10$). From the shift of f_c^A with

Table 1 The rate constant k_1' and the factor G in equation (2) which are approximately independent of $[Na]_o$

$[Na]_o$ (mM)	k_1' ($\mu M^{-1}\ s^{-1}$)	$G \times 10^3$ (s) $[A]_o = 4$ μM	$[A]_o = 8$ μM
6	13.6 ± 0.4	14.8 ± 1.4	7.9 ± 0.5
20	12.4 ± 0.5	14.2 ± 1.2	8.3 ± 0.6
30	13.0 ± 0.6	12.7 ± 0.5	7.9 ± 0.3
45	12.5 ± 0.4	12.0 ± 0.5	6.7 ± 0.2
60	12.2 ± 0.3	13.0 ± 0.7	7.5 ± 0.5

G was calculated for two values of $[A]_o$. Each value corresponds to the mean ± s.e.m. of six observations.

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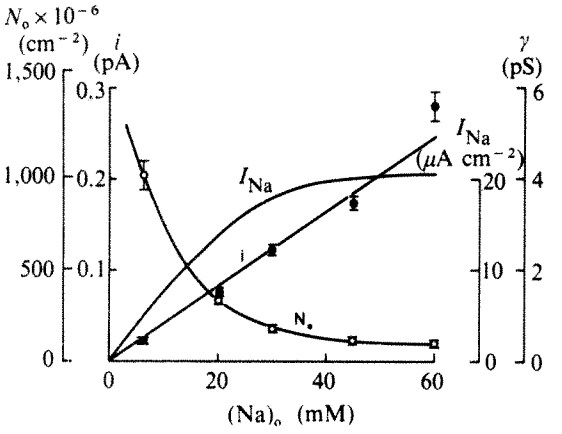


Fig. 2 ●, Computed values (equation (2)) of Na^+ current (i) through single conducting pores at different outer Na^+ activities. Spectra obtained with an amiloride concentration of 6 μM were used ($n = 7$). Bars represent s.e.m. The conductance (pS) of single open pores was computed using equation (3). ○, The densities of conducting pores (N_o). The curve labelled I_{Na} represents the total amiloride-sensitive Na^+ current which passed 1 cm^2 of epithelium in the absence of amiloride in the experiment represented by the data points.

$[A]_o$ (equation (1)) we calculated, for different sodium activities, tentative values for k_1', k_2 and the apparent dissociation constant K_A'. k_1', which is the slope of the straight line through the data points of the rate/concentration plot, could be determined quite accurately. However, there was a greater experimental variation in k_2 and K_A', which are, respectively, the intercept with the ordinate and abscissa. Table 1 lists the values for k_1' obtained at different Na^+ concentrations. k_1' does not change significantly with $[Na]_o$, whereas the apparent value of k_2 increases with $[Na]_o$. The apparent mean lifetime of the amiloride–receptor complex calculated from these k_2 values was 75 ± 2 ms and 382 ± 75 ms, respectively, at $[Na]_o$ 60 mM ($n = 61$) and 20 mM ($n = 24$).

The current, i, passing through single open pores was calculated from Lorentz spectra using

$$i = S_o^{\wedge}/(4aGI_{Na}) \qquad (2)$$

where $G = (2\pi f_c^{\wedge})^{-2} \times k_1' \times [A]_o$. Equation (2) is equivalent to equation (6) derived in ref. 5. a denotes the membrane area and I_{Na} the mean net Na^+ current per cm^2 observed while current fluctuations were being registered. We found that $S_o^{\wedge}$ and I_{Na} changed with $[Na]_o$, whereas k_1' and $f_c^{\wedge}$ remained almost constant. Therefore, the factor G remains approximately constant as long as $[A]_o$ does not change (see Table 1). The computed values of i are shown in Fig. 2 as a function of $[Na]_o$. The current through single conducting pores can be seen to increase linearly with the outer Na^+ activity, although the mean net Na^+ current becomes saturated (Fig. 2, curve labelled I_{Na}). This result supports the previous conclusion[2] that the transport capacity of individual channels (pores) cannot be responsible for the saturation of net Na^+ uptake with increasing $[Na]_o$.

The change of i with $[Na]_o$ enables the conductance of single open pores to be calculated. The current–voltage curve of the Na^+-selective membrane can be predicted by the constant field equation[2]. Assuming the intracellular Na^+ concentration to be negligible relative to $[Na]_o$ in the amiloride concentration range used, the slope conductance of the single open pore (γ) can be approximately estimated at zero voltage across the apical membrane as

$$\gamma = \frac{i}{2RT/F} \qquad (3)$$

R, T and F have their usual meaning. γ is thus proportional to i, and so increases linearly with $[Na]_o$. The appropriate ordinate scale for γ has been included in Fig. 2. Mean values of 5.5 pS are reached at $[Na]_o$ values of 60 mM.

Knowing the dependence of i on $[Na]_o$, the expected change in the density of conducting pores, N_o, with $[Na]_o$ can be calculated from the expression $N_o = I_{Na}/i$. Using this, we do, indeed, find that N_o decreases with increasing $[Na]_o$ (Fig. 2), thus confirming our hypothesis that the saturation of Na^+ transport with increasing $[Na]_o$ is caused by a decrease in the number of conducting translocators. This regulatory 'inactivation' of transporting channels may prevent Na^+ overloading of the epithelial cells in situations in which there is a large environmental Na^+ supply.

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Human erythrocyte anion exchange site characterised using a fluorescent probe

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A plasma membrane anion exchange system allows the red blood cell to replace intracellular bicarbonate rapidly with extracellular chloride to facilitate carbon dioxide release into the lungs. The protein responsible for this exchange is a glycoprotein of molecular weight about 93,000, called band 3, which comprises approximately 25% of the total red cell membrane protein and is present in the membrane as a non-covalent dimer[1]. The molecular mechanism of anion transport remains obscure, in spite of the characterisation of the extracellular anion binding site by a number of methods including interactions with disulphonic stilbene probes which act as specific inhibitors of anion exchange[2]. One of these compounds (4,4'-diisothiocyano-2,2'-disulphonic stilbene; DIDS) causes complete inhibition of anion exchange by reacting at approximately one site per band 3 monomer (~2.5 nmol per mg protein)[3]. A fluorescent DIDS analogue, DBDS (4,4'-dibenzoamide-2,2'-disulphonic stilbene), binds reversibly and specifically to the DIDS reactive site where it also inhibits anion exchange[6]. When bound, the fluorescence intensity of DBDS increases by two orders of magnitude and this has enabled us to investigate the binding kinetics of DBDS to red blood cell ghost membranes by fluorescence equilibrium and temperature-jump methods. Our equilibrium binding data indicate that there are two classes of DBDS binding sites on red cell ghosts. The reaction scheme that we present here suggests that the apparent high affinity (15–60 nM) for binding the first DBDS molecule is the result of a two step process: an initial binding of lower affinity (K_1~0.5 μM) to band 3 followed by a slow conformational change (~5 s^{-1}) in the binding site which acts to lock the DBDS molecule in place. A second DBDS molecule binds with low affinity (~3 μM) to a site which is more difficult to characterise.

The equilibrium binding affinities for DBDS binding to red cell ghosts were determined as described in Fig. 1 legend. All equilibrium and relaxation experiments were conducted in 28.5 mM sodium citrate, pH 7.4 (160 mM ionic strength). No transportable anions were present so that the system could be

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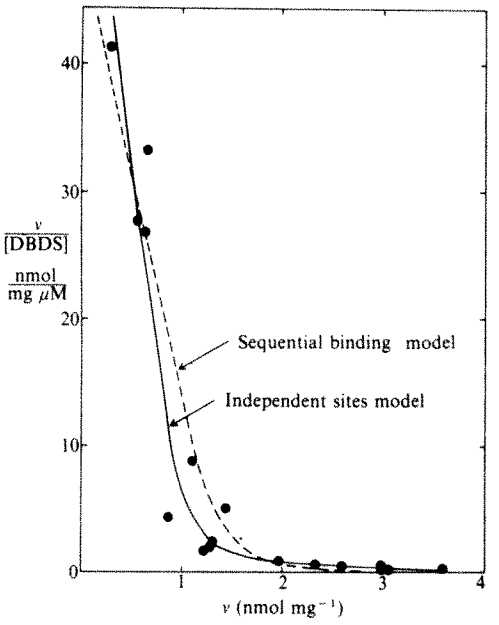


Fig. 1 A Scatchard plot of DBDS binding to red cell ghosts. ν is the DBDS (nmol) bound per mg of ghost protein and [DBDS] is the concentration of unbound DBDS. Ghosts (prepared by standard procedures[4]) were incubated with known concentrations of DBDS for 1 h in 28.5 mM sodium citrate, *p*H 7.4, 25 °C and then centrifuged at 40,000*g* for 15 min. The concentration of DBDS in the supernatant was determined by absorbance at 336 nm or by fluorescence (excitation 336 nm, emission 435 nm). The quantity of DBDS bound was determined from the difference between total and free. The ghost concentration (determined by the Lowry[5] procedure) varied between 0.4 and 1.3 mg ml^{-1}. The solid line was drawn using the equation: $\nu/[DBDS] = n_1/([DBDS]+K_1') + n_2/([DBDS]+K_2')$ which assumes two independent binding sites with $n_1 = 1.0$ nmol mg^{-1}, $K_1' = 16$ nM and $n_2 = 2.7$ nmol mg^{-1}, $K_2' = 4.4$ μM. The dotted line was drawn using the equation: $\nu/[DBDS] = 2n(K_2^a + [DBDS])/(K_1^a K_2^a + 2[DBDS](K_2^a + [DBDS]/2))$ which assumes two identical interacting sites, each with stoichiometry $n = 1.36$ nmol mg^{-1} and with intrinsic dissociation constants for the first and second ligand of $K_1^a = 56$ nM and $K_2^a = 1.86$ μM.

studied in simple conditions. The curved Scatchard plot in Fig. 1 can be explained on the basis of two classes of independent sites (solid line) or negative cooperativity in DBDS binding to identical sites (dashed line). The high affinity DBDS binding may be completely eliminated by reacting whole cells with DIDS before ghost cell preparation[6]. However, the maximum specific incorporation of DIDS (or BIDS[6]; 4-benzoamido-4'-isothiocyano-2,2'-disulphonic stilbene) into band 3 is approximately 2.5 nmol per mg ghost protein while the stoichiometry of the apparent high affinity site ($K_1' = 16$ nM, independent binding site model) is only 1–1.3 nmol per mg. It is not clear how much, if any, of the low affinity binding is on band 3. Equilibrium binding affinities determined by fluorescence enhancement (data not shown) closely paralleled the results shown in Fig. 1.

The rate constants for DBDS equilibration with the ghost membrane were determined by the temperature-jump method[7]. As illustrated in Fig. 2, a rapid decrease in DBDS fluorescence occurred during the instrument heating time (~5 μs) corresponding to a rapid decrease in the quantum yield of DBDS; this was followed by a slower exponential decay in fluorescence on the millisecond time scale. The slow decay was not observed with sonicated lipids extracted from red cell ghosts and the amplitude (corrected for inner filter effects) of the relaxation decreased by over 95% after incubation with 4 μM DIDS indicating that the fluorescence decrease is primarily due to the release of DBDS from band 3.

The concentration dependence of the exponential relaxation is presented in Fig. 3*a*. At high concentrations of free DBDS, the apparent rate constant ($1/\tau$) becomes independent of concentration. These data require a minimal model consisting of a single bimolecular step in which DBDS rapidly binds to its receptor, R, followed by a slow concentration-independent conformational change:

$$DBDS + R \overset{K_1}{\longleftrightarrow} R{\cdot}DBDS \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} R'{\cdot}DBDS$$

The relaxation expression for this model is given in the legend to Fig. 3*a*. The straight line in the inset is the best fit subject to the constraint that the equilibrium dissociation constant is 16 nM as determined for the high affinity site of the independent site model in Fig. 1 ($K_1' = K_1/[1+(k_2/k_{-2})] = 16$ nM); the free ligand and site concentrations are given by the equilibrium data. The model did not fit the data well at low DBDS concentrations and, if K_1' was not constrained, k_{-2} became negative.

We therefore explored a model in which a second DBDS molecule is assumed to bind rapidly but weakly to the receptor following the conformational change:

$$R \overset{K_1}{\longleftrightarrow} R{\cdot}DBDS \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} R'{\cdot}DBDS \overset{K_3}{\longleftrightarrow} R'{\cdot}(DBDS)_2$$
$$\qquad\qquad\qquad

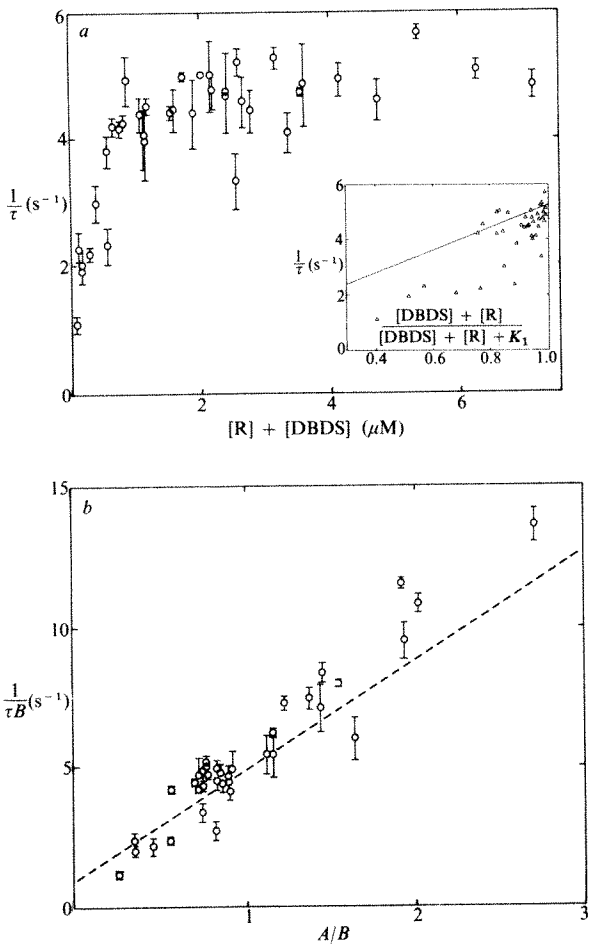


Fig. 3 *a*, Kinetics of DBDS binding to band 3. The abscissa values, the sum of the free concentrations of DBDS and receptor, are determined from the equilibrium binding data shown in Fig. 1 for the independent site model. The ordinate, $1/\tau$, is the reciprocal time constant obtained from a fit to relaxation data. Each data point represents an average of four experiments; error bars indicate one standard deviation from the mean. These data require a minimal mechanism consisting of a rapid bimolecular step followed by a slower conformational change. The relaxation expression for this model (see inset) is $1/\tau = k_{-2} + k_2([DBDS]+[R])/(K_1+[DBDS]+[R])$, where [DBDS] and [R] are the free concentrations of DBDS and receptor sites calculated from the high affinity independent site model binding data of Fig. 1 and the constants defined in the text. The solid line is the best fit using the assumptions in the text with $k_{-2} = 2.3\ s^{-1}$ and $k_2 = 3.3\ s^{-1}$. The effect of the low affinity independent site on the relaxation times predicted by this minimal model have been neglected for simplicity. A fit which includes the effect of the second binding site is similar to that obtained with the minimal model[10]. *b*, A fit of the relaxation expression for the three step model to the experimental data. Assuming rapid equilibrium for the two binding steps, K_1 and K_3, the relaxation rate as determined by standard procedures[9] is $1/\tau = k_2A + k_{-2}B$ where:

$$A = 1 - \frac{yK_1/2 - [R][DBDS]}{xy - 2[R]^2[DBDS]/K_1K_2}$$

$$B = 1 - \frac{x[DBDS] - [R][DBDS]/K_2}{xy - 2[R]^2[DBDS]/K_1K_2}$$

$$x = [R] + [DBDS] + K_1/2$$

$$y = [DBDS](1 + 2[R]/K_1K_2) + 2K_3$$

$$K_2 = k_{-2}/k_2$$

The solid line through the data is the best fit ($r = 0.94$) with the constraints that $K_1 = K_1^a(1 + k_2/k_{-2}) = 56\ nM(1 + k_2/k_2)$ and $K_3 = K_2^a/(1 + k_{-2}/k_2) = 1.86\ \mu M/(1 + k_2/k_2)$ as determined from the negative cooperativity model in Fig. 1. [R] and [DBDS] are the concentrations of unoccupied sites and free DBDS as determined from the data in Fig. 1. The values determined for k_2 and k_{-2} were $4.4\ s^{-1}$ and $1.0\ s^{-1}$ respectively.

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locks the first DBDS molecule to the receptor site; subsequently a second DBDS molecule binds to a second site with lower affinity. These studies also illustrate the value of the temperature-jump technique in examining the detailed mechanism of membrane transport reactions.

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Distribution of light chains in fast skeletal myosin

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Myosin is composed of two heavy chains of molecular weight 200,000 and four light chains of approximate molecular weight 20,000. The heavy chains are organised into a two-headed structure of which each head is completed by the presence of two light chains[1,2]. In fast skeletal myosin the light chains can be divided into two classes which are chemically and functionally distinct. One member of each class is associated with each head. The class known as the alkali light chain contains two related variants: alkali 1 (A1) and alkali 2 (A2). These light chains are identical in amino acid sequence over their C-terminal 141 residues, but differ in their N-terminal sequences[3]. The non-integral stoichiometry of light chains in rabbit skeletal myosin[4,5] first suggested that at least some myosins must be homodimeric (a molecule with the same light chain on each head), but it was not clear to what extent heterodimers (molecules containing both alkali 1 and alkali 2 light chains) also occurred. The most direct approach to determining the distribution of light chains in myosin is to isolate the different myosin isoenzymes. Although ion-exchange chromatography has been successful in separating the single-headed sub-fragments of myosin[6], it has not been possible to fractionate double-headed species except by some type of affinity chromatography[7,8]. We have shown that homodimers of myosin can be isolated from chicken pectoralis myosin by immunological methods[9,10]. By comparing the electrophoretic patterns of homodimeric molecules with whole myosin in non-denaturing gels, it has been possible to demonstrate conclusively the existence of a light chain heterodimer.

Antibodies specific for the N-terminal sequence of alkali 1 or alkali 2 can be used as immunoadsorbents to fractionate heavy meromyosin or myosin into two components[9,10]. When myosin is passed through an affinity column prepared by coupling antibody to Sepharose, a fraction rich in alkali 1 (Fig. 1*b*) or alkali 2 (Fig. 1*c*) is obtained in the void volume, depending on the specificity of the column. The retained myosin fraction can only be eluted by a denaturant such as guanidine hydrochloride, or a weak acid. It is evident that the isolated homodimers are both slightly contaminated by the heterologous light chain: that is, A1 myosin contains about 10% alkali 2 light chain (Fig. 1*b*) and A2 myosin is contaminated by a similar amount of alkali 1 light chain (Fig. 1*c*). The antibody-bound myosin (not shown

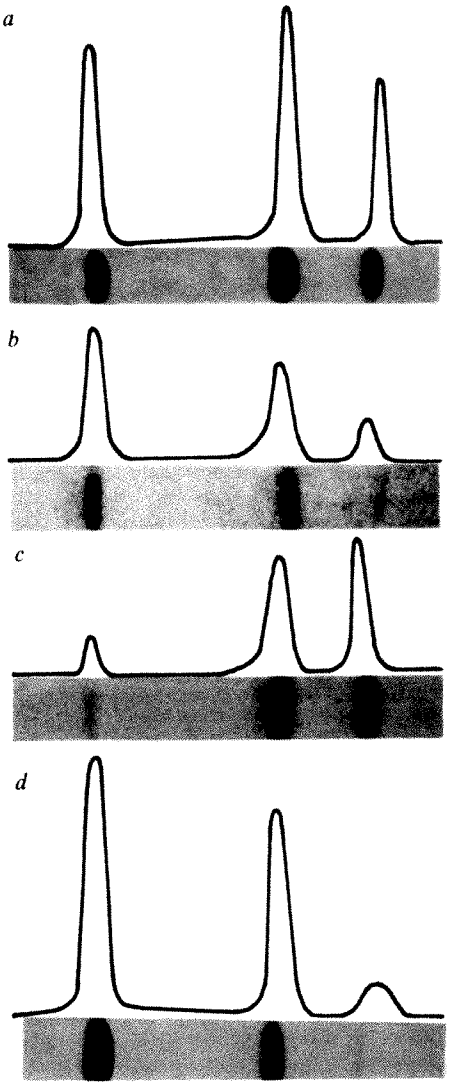


Fig. 1 SDS polyacrylamide gel electrophoresis of chicken pectoralis myosin. A separating gel 12.5% in acrylamide (*p*H 8.8) and a stacking gel 5% in acrylamide (*p*H 6.8) was used according to the method of Laemmli[24]. Gels were stained in 0.025% Coomassie brilliant blue (R250) dissolved in 45% methanol and 9% acetic acid, and destained by diffusion in 5% methanol and 7.5% acetic acid. Myosin loads are about 10 μg. Electrophoresis is from left to right (positive electrode) separating light chains into Al, DTNB and A2, respectively. *a*, Myosin control; *b*, Al myosin; and *c*, A2 myosin were prepared from chicken pectoralis myosin by using immunoadsorbents specific for A2 light chain and Al light chain, respectively (see refs 9 and 10); *d*, A1-exchanged myosin was prepared from chicken pectoralis myosin by addition of a tenfold molar excess of Al light chain in the presence of 4.7 M NH_4Cl, as described by Wagner and Weeds[15]. Excess light chain was removed by gel filtration on Sephacryl S-200 (Pharmacia). Gels were traced on a Joyce Lobel double-beam recording microdensitometer.

here, see refs 9 and 10), is contaminated by an even greater amount of heterologous light chain. If we assume that all the heterologous light chain in both the retained and unretained fractions is derived from a heterodimeric species, a maximum estimate of about 30% A1, A2 myosin is calculated[11]. However, any nonspecific binding of myosin to these immunoadsorbents would result in an overestimate of this figure. To establish with any confidence that a significant proportion of the myosin population consists of heterodimer, it was necessary to apply an independent approach to this problem.

The studies of Hoh *et al.* have provided such an alternative method by demonstrating that myosin solubilised in alkaline pyrophosphate buffer can be fractionated in low percentage polyacrylamide gels in the complete absence of denaturing agents[12]. In such a system, where myosin migrates according to its intrinsic mobility at that *p*H, chicken pectoralis myosin was found to separate into three bands. The material from each band was isolated by slicing the gel, and the light chain pattern was analysed by SDS polyacrylamide gel electrophoresis. It was found that the least mobile band contained only alkali 1 light chain, the most mobile band had only alkali 2 light chain, and the middle band contained both light chains. These results suggested that the band of intermediate mobility might be a light chain heterodimer[13,14]. However, it was not clear to what extent heavy chain heterogeneity might contribute to the band separation in this system, and it was possible that the band of intermediate mobility might arise from a mixture of light chain homodimers with different heavy chains. Our ability to prepare relatively pure homodimers of myosin allowed us to investigate directly whether the heavy chains contribute to the mobility of the isoenzymic species.

Figure 2 shows non-denaturing gels of native chicken pectoralis myosin and the homodimeric myosin molecules isolated by immunoadsorption as described above. The unfractionated myosin shows three bands (Fig. 2*a*), whereas A1 myosin (Fig. 2*b*) and A2 myosin (Fig. 2*c*) each migrate as a single band. Moreover, the A1 isoenzyme co-migrates with the slowest moving component in the control (Fig. 3*b*) and the A2 isoenzyme co-migrates with the fastest species (Fig. 3*c*). To demonstrate that the intermediate band was not generated by light chain exchange in the slightly alkaline conditions of electrophoresis, A1 and A2 myosin were mixed and applied to one gel. No intermediate band was observed (Fig. 2*d*). Similarly, no intermediate band was generated when the two isoenzymes were dialysed together for 24 h against pyrophosphate electrophoresis buffer. It thus seems reasonable to conclude that the band of intermediate mobility represents a light chain heterodimer, and is not simply the result of heavy chain heterogeneity or an experimental artefact.

These experiments do not rule out the possibility that the heavy chains contribute to the mobility of the individual myosin isoenzymes in this gel system. If, however, myosin mobility is solely a function of the alkali light chain, it should be possible to generate a single electrophoretic species from native pectoralis myosin by exchanging the native light chains with either alkali 1 or alkali 2. Such an exchange has been shown to occur in myosin subfragment-1 when it is incubated with an excess of alkali light

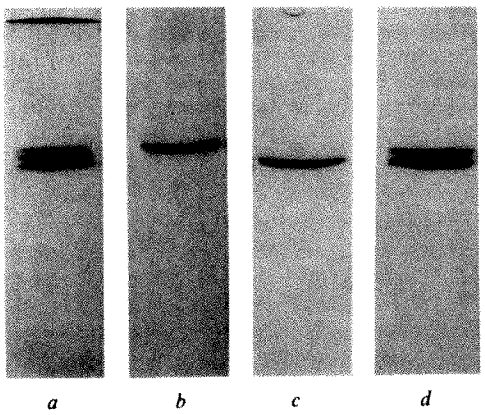


Fig. 2 Native myosin on non-denaturing gels. Polyacrylamide gels (4% acrylamide, 3% bisacrylamide) were prepared in the absence of denaturing agents in 40 mM pyrophosphate buffer (*p*H 8.7), 1 mM EDTA, 2 mM cysteine, 10% glycerol according to the method of Hoh[12]. Electrophoresis was carried out at a constant current of 6 mA per gel for 16 h with buffer recirculating at 60 ml per min. Gels were stained in 0.04% Coomassie brilliant blue (G250) dissolved in 3.5% perchloric acid[25], and destained by diffusion in 5% methanol and 7.5% acetic acid. Myosin loads range from 1 to 3 μg. Positive electrode was at the bottom of the gel. Samples were prepared as described in Fig. 1. *a*, Myosin control is resolved into A1 homodimer, A1,A2 heterodimer, and A2 homodimer (see text); *b*, A1 homodimer, and *c*, A2 homodimer each electrophorese as a single band, while the mixture (*d*) of both isoenzymes resolves into a two-band pattern lacking the middle band found in control myosin.

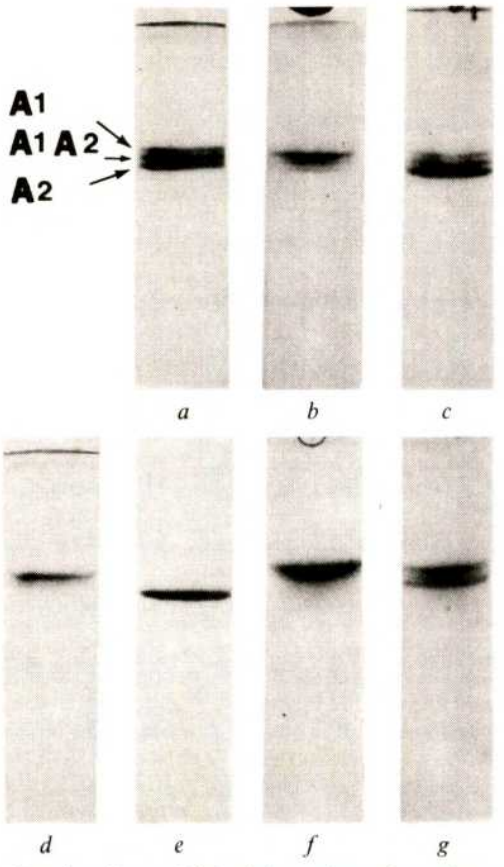


Fig. 3 Co-migrations and light chain-exchanged myosins on non-denaturing gels. Gels were run as previously described in Fig. 2. *a*, Control myosin shows three bands of approximately equal intensity; *b*, impure sample of myosin eluted in the void volume from an antibody affinity column specific for A2 light chain. There is an enrichment of the band of slowest mobility indicating that this contains A1 homodimer. *c*, Co-migration of control myosin and A2 myosin isolated from an antibody affinity column specific for A1 light chain. The enrichment of the band of fastest mobility shows that this represents A2 homodimer. Exchange of total (control) myosin with a tenfold molar excess of A1 light chain in 4.7 M NH_4Cl resulted in myosin of uniform mobility (*d*) which co-migrated with the single band from A1 myosin. A similar exchange was carried out using A2 myosin as the starting material (*e*) and adding an excess of A1 light chain in 4.7 M NH_4Cl. The exchanged myosin again ran as a single band (*f*) with a shift in mobility from that of the A2 myosin. Co-migration of A1-exchanged myosin and A2 myosin (*g*) resulted in two bands.

chain in the presence of 4.7 M NH_4Cl (ref. 15). By this means, a myosin species was generated from total chicken pectoralis myosin which contained only the alkali 1 light chain as determined by SDS gel electrophoresis (Fig. 1*d*). Electrophoresis of this myosin in the non-denaturing gel system shows primarily a slow-moving A1 myosin component, and trace amounts of the band of intermediate mobility (Fig. 3*d*). A similar result was obtained by exchanging A2 myosin homodimer produced by antibody affinity chromatography (Fig. 3*e*) with A1 light chain. A myosin species was generated whose mobility (Fig. 3*f*) was indistinguishable from that of naturally occurring A1 myosin homodimer.

The results presented here strongly suggest that in chicken pectoralis myosin the alkali light chain content is the principal determinant of mobility differences on non-denaturing polyacrylamide gels in pyrophosphate buffer. Therefore, the band of intermediate mobility must be an alkali light chain heterodimer, as suggested[13,14]. Chicken pectoralis myosin contains approximately equal amounts of alkali 1 and alkali 2 light chains[9,11]. If heavy chains associated randomly with light chains, one would predict that 50% of the myosin from this muscle would be heterodimeric for the light chains, 25% homodimeric for alkali 1 and 25% homodimeric for alkali 2. It is difficult to quantify the relative amounts of each component from densitometer traces of the gels shown in Fig. 2, as the resolution is poor, but we would estimate that there are approximately equal amounts of each species. A similar estimate has been made from pyrophosphate gel densitometry by Hoh[13] and d'Albis[14], and from quantification of material eluted from antibody affinity columns[11]. These values suggest, therefore, that light chain–heavy chain association is not strictly random.

Heavy chain heterogeneity has been observed in certain peptides isolated from rabbit myosin[16,17]; however, both of the heavy chain *N*-terminal peptides were found to be associated with each of the two subfragment-1 isoenzymes[18]. These findings, together with the observation that the actin-activated ATPase activity appears to depend on the type of alkali light chain present[15], argue against a specific interaction between the light and heavy chains of myosin. Nevertheless, these data cannot exclude the possibility that the alkali light chain homodimers may yet prove to differ from each other in heavy chain sequence, in spite of the absence of a contribution by the heavy chain to the electrophoretic mobility.

Precedents exist for both heavy chain homodimers and heterodimers. In chicken anterior latissimus dorsi myosin[13], and rat ventricular myosin[19], multiple band patterns on pyrophosphate gels have been demonstrated, in spite of the existence of only one light chain species analogous to the alkali light chains. In the case of rat ventricular myosin, peptide mapping of three isolated species showed the presence of two types of heavy chain homodimers as well as a heterodimeric myosin[19].

Homodimers of heavy chains have also been shown to occur in the nematode worm *Caenorhabditis elegans*[20]. Moreover, the synthesis of the two types of heavy chains is coordinated throughout larval development, and reflects the final accumulation of two populations of homodimeric molecules[21]. No heterodimers are detectable in the nematode. In contrast, the alkali 1 and alkali 2 light chains are not simultaneously synthesised during early development of vertebrate skeletal muscles and alkali 2 is largely absent in the prenatal stages of the rat[22] and chicken[23]. It is possible that uncoordinated synthesis of the alkali light chains persists in adult muscle cells, and this may account for the non-random distribution of light chains among the isoenzyme species. Alternatively, the affinity of the light chains for the heavy chains may be such that the formation of a heterodimer is less favoured than the more symmetrical molecules.

Although no single satisfactory explanation can be offered for the occurrence of light chain homodimers and heterodimers in fast skeletal myosin, the proof of their existence at least raises the intriguing question of their function in muscle contraction.

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Nucleotide sequence of a cDNA clone encoding human preproinsulin

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Insulin consists of two polypeptide chains, A (21 amino acids) and B (30 amino acids), linked by disulphide bonds. Both chains are derived from one precursor, proinsulin, which includes a connecting peptide (C) between the A and B chains, and which is excised before the secretion of insulin from the pancreatic B cells[1]. The observation that the C-peptide varies between species, in contrast to the highly conserved A and B sequences[1-3], is consistent with the theory that it serves a purely structural function in insulin synthesis. During *in vitro* translation of insulin mRNA, a larger peptide containing about 25 additional residues at the N-terminal end (preproinsulin) is the primary product[4-7]. The prepeptide is cleaved to leave proinsulin during transport into the endoplasmic reticulum and is thought to direct this process specifically[8]. Using automated amino acid sequence analysis, the partial amino acid sequences of the prepeptide regions of bovine, rat, sea raven and anglerfish preproinsulin have been established[4,5,7]. The nucleotide sequences of the cloned cDNA and gene coding for rat insulin I have confirmed the amino acid sequence of rat proinsulin I, and have also predicted the sequence of the prepeptide[9-11]. Like the prepeptides of other secreted proteins, this prepeptide has a prominent hydrophobic region[12]. We report here the cloning of a cDNA prepared from human insulin mRNA and an analysis of the nucleotide sequence of the cloned molecule including the region coding for the prepeptide and portions of the 5'- and the 3'-untranslated regions of the molecule. We also compare the structure of the human molecule with the previously reported rat mRNA[9-11].

The mRNA used in these experiments was prepared from a human insulinoma by the guanidinium thiocyanate procedure[9]. Approximately 40 ng of double-stranded cDNA was prepared from 130 μg of unfractionated RNA and inserted into the *PstI* endonuclease site of the β-lactamase gene of pBR322 (ref. 13) using poly(dG)·poly(dC) homopolymeric extensions. The *Escherichia coli* strain X1776 was transformed with this DNA in P2 containment conditions according to the NIH Guidelines. This produced 525 tetracycline-resistant transformants. These were replica-plated and screened for insulin sequences by a modification of the colony hybridisation method of Grunstein and Hogness[14] using nick translated cloned rat preproinsulin I cDNA[9,11] as a probe. Since the amino acid sequence of rat and human insulins are quite similar (insulin, 92% homology and proinsulin, 83% homology), it was anticipated that the cloned rat cDNA should cross-hybridise with human insulin sequences in conditions of reduced stringency. After hybridisation as described[11], the filters were washed in a solution of 0.015 M sodium chloride, 0.0015 M sodium citrate, 0.1% SDS at 42 °C for 1 h. Autoradiography revealed that 2 out of the 525 colonies hybridised with the cloned rat preproinsulin I probe. Both colonies were ampicillin sensitive and contained plasmids that were approximately 250 and 500 base pairs larger than pBR322, the plasmid vector. The larger plasmid, pcHI-1, was analysed further.

The nucleotide sequence of the inserted cDNA fragment was determined by the procedure of Maxam and Gilbert[15]. The overall structure of the cDNA clone pcHI-1 and the sequencing strategy used are depicted in Fig. 1. The nucleotide sequence of both DNA strands was determined as indicated and the sequence of the remaining portions were done in duplicate; in all cases sufficient data were obtained to overlap the junctions of the fragments. The mRNA sequence derived from the cDNA

```
                -24              -20
            met ala leu trp met arg leu leu pro leu leu ala leu
UCCUUCUGCC  AUG GCC CUG UGG AUG CGC CUC CUG CCC CUG CUG GCG CUG

    -10                                              1
leu ala leu trp gly pro asp pro ala ala ala phe val asn gln his
CUG GCC CUC UGG GGA CCU GAC CCA GCC GCA GCC UUU GUG AAC CAA CAC

                  10                                          20
leu cys gly ser his leu val glu ala leu tyr leu val cys gly glu
CUG UGC GGC UCA CAC CUG GUG GAA GCU CUC UAC CUA GUG UGC GGG GAA

                                  30
arg gly phe phe tyr thr pro lys thr arg arg glu ala glu asp leu
CGA GGC UUC UUC UAC ACA CCC AAG ACC CGC CGG GAG GCA GAG GAC CUG

          40                                              50
gln val gly gln val glu leu gly gly gly pro gly ala gly ser leu
CAG GUG GGG CAG GUG GAG CUG GGC GGG GGC CCU GGU GCA GGC AGC CUG

                            60
gln pro leu ala leu glu gly ser leu gln lys arg gly ile val glu
CAG CCC UUG GCC CUG GAG GGG UCC CUG CAG AAG CGU GGC AUU GUG GAA

 70                                              80
gln cys cys thr ser ile cys ser leu tyr gln leu glu asn tyr cys
CAA UGC UGU ACC AGC AUC UGC UCC CUC UAC CAG CUG GAG AAC UAC UGC

 86
asn AM
AAC UAG  ACGCAGCCCGCAGGCAGCCCCCCACCCGCCGCCUCCUGCACCGAGAGAGAUGGA

AUAAAGCCCUUGAACCAGC
```

Fig. 2 Sequence of human preproinsulin mRNA. The sequence of the mRNA was deduced from that of the cDNA insert in pcHI-1. The predicted amino acid sequence is indicated above. The prepeptide region extends from the Met at position −24 to the Ala at position −1, the B-peptide from the Phe at position 1 to the Thr at position 30, the C-peptide from the Arg at position 31 to the Arg at position 65, and the A peptide from the Gly at position 66 to the Asn at 86. This cDNA insert also contains a poly(A) tract of approximately 45 bases extending from the 3'-terminal cytosine.

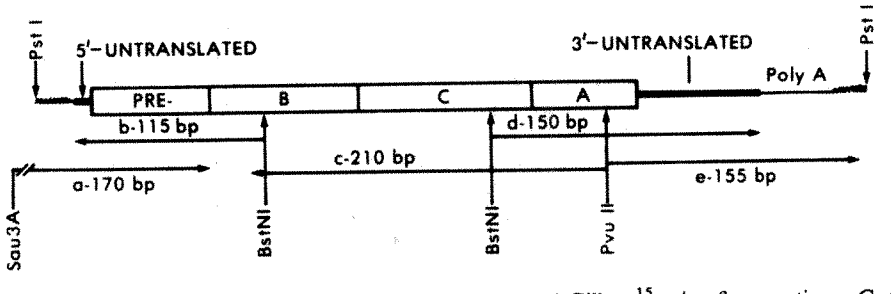


Fig. 1 Organisation and sequence strategy for the human preproinsulin cDNA insert in pcHI-1. The regions coding for preproinsulin are indicated by the open boxes. The wavy lines indicate the poly(dG)·poly(dC) tails of the cDNA insert (27 base pairs at the 5' end and 16 base pairs at the 3' end of the cDNA insert, respectively). Only the restriction sites used for sequencing are indicated. The 5'-end-labelled restriction fragments were derived from the intact plasmid and after the appropriate secondary endonuclease digestion used for sequencing by the procedure of Maxam and Gilbert[15] using five reactions: *G*, *G*+*A*, *A*>*C*, *T*+*C*, *C*. The number of bases determined from each sequencing experiment starting at the indicated restriction site is shown above the arrow. The *Sau*3A site is in the β-lactamase gene 58 base pairs from the *Pst*(I) site[13]. The size of human preproinsulin mRNA is approximately 550 base pairs as determined by hybridisation after electrophoresis in CH_3HgOH–agarose gels[16]. The cloned cDNA (cHI-1) is only 461 base pairs (including 45 A residues) and thus does not represent a complete copy of the mRNA.

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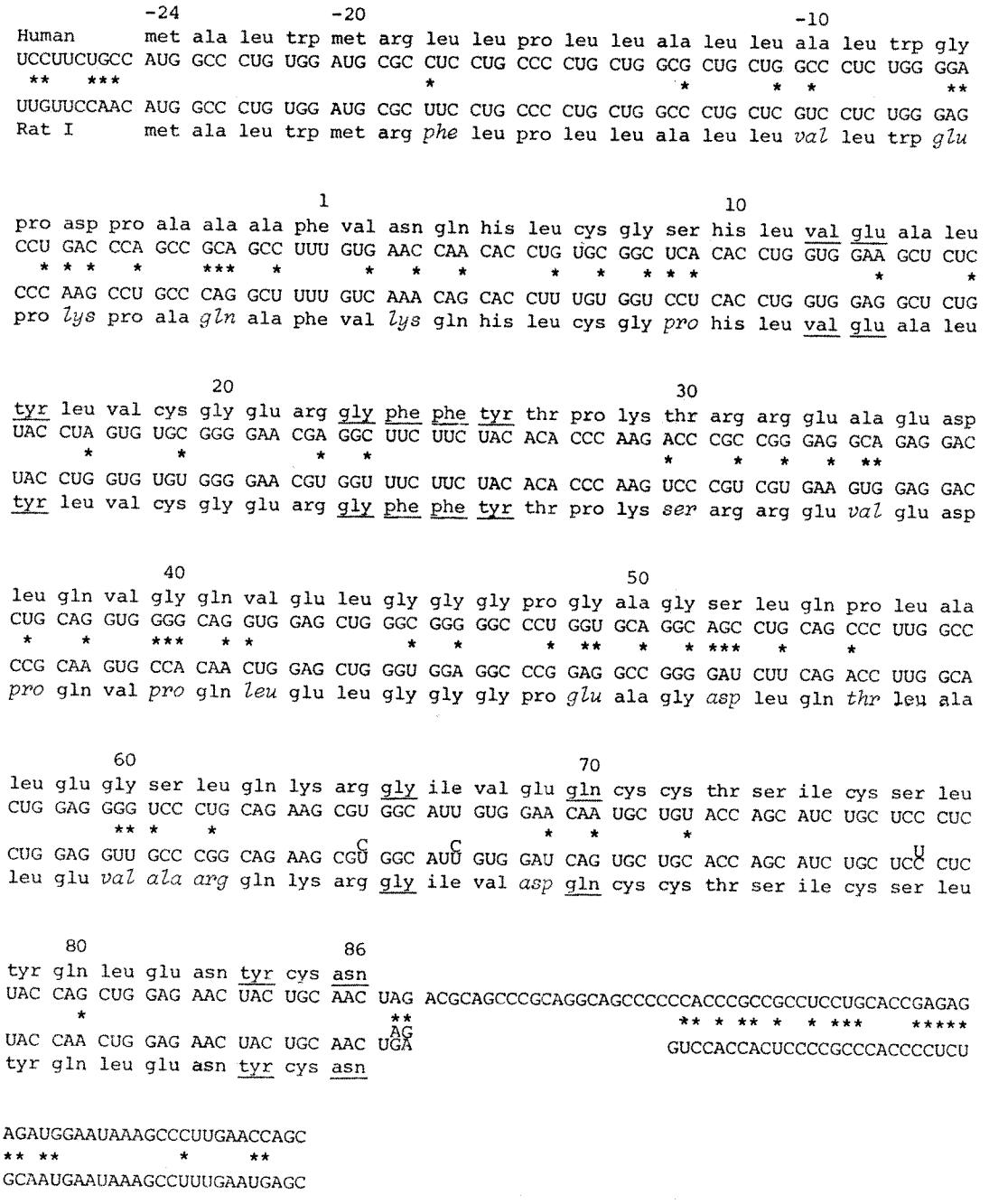


Fig. 3 Comparison of the sequence of human and rat preproinsulins. The sequence previously determined for rat preproinsulin I mRNA is indicated below that of human preproinsulin mRNA[9–11]. The nucleotide differences are indicated by asterisks. The amino acid sequence of the rat preproinsulin[9–11] is also compared with that of human preproinsulin. The differences in amino acids are indicated by italics. The amino acid residues believed to be involved in insulin receptor binding are underlined[17]. The 3′-untranslated region of rat insulin I has been shifted so as to emphasise its homology with human insulin mRNA in the 21-base pair region adjacent to the poly(A) attachment site. The previously cloned sequence of rat insulin II extends from the third base of the arginine codon at amino acid 62 to the G residue following the termination codon UAG. The nucleotide changes in the A chain of rat insulin II are indicated above the rat insulin I mRNA sequence; they result in three additional third-base changes; the termination codon for rat II is the same as for human insulin.

sequence, and the amino acid sequence predicted from one of the reading frames is presented in Fig. 2. The primary structure of human proinsulin determined in this manner agrees precisely with that obtained by previous amino acid sequencing experiments[2,3]. The predicted 24-amino acid sequence of the human prepeptide is similar to the sequence of rat insulin I and to the partial sequences obtained from bovine, sea raven and anglerfish prepeptides[4,5,7,9,11]. The nucleotide sequence of a small portion (10 bases) of the 5′-untranslated region and the complete sequence (76 bases) of the 3′-untranslated region (including the poly(A)attachment site) were also obtained.

Although the human preproinsulin cDNA is inserted into the β-lactamase gene of pBR322 in the correct orientation for expression, we would not expect a fused β-lactamase human preproinsulin polypeptide to be synthesised, since the two sequences are in different reading frames. We have examined the polypeptides produced in minicells by this plasmid (S. C. Rall and G.I.B., unpublished observations) and observed no polypeptides of the size expected for the fusion protein (approximately 300 amino acids). Instead, a molecule of approximately 220 amino acids was obtained. The nucleotide sequence predicts that translation of the β-lactamase–cDNA chimaeric mRNA should produce a fused protein which terminates at the UGA sequence at the junction of the second and third amino acids of the insulin B chain (Val·Asn, G*UG·AA*C; Fig. 2). This would generate a protein of the size observed.

The structure of insulin is highly conserved[1–3]. We have therefore compared the amino acid and nucleotide coding sequences, as well as the adjacent regions, of the mRNAs of human and rat insulin I (Fig. 3). The nucleotide sequence of the A chain of rat insulin II is also included[9]. This analysis is summarised in Table 1.

The high degree of amino acid sequence homology of the A and B chains extends to the nucleotide sequence. This is evident throughout the A chain and most of the B chain. However, in the B chain there are considerable nucleotide changes, largely in the third positions of the codons; thus there is more homology at the amino acid than at the nucleotide level. This contrasts with the situation in human and rat pregrowth hormones where the nucleotide homology is higher than the amino acid homologies[18–20]. A number of the amino acid residues are invariant in all insulin molecules and are believed to be involved in receptor

Table 1 Comparison of human and rat I and II preproinsulins and their mRNAs

Region	Homology (relative to human) Amino Acid Rat I	Amino Acid Rat II	Nucleotide Rat I	Nucleotide Rat II
5'-Untranslated	—	—	5/10 (50%)	—
Pre-peptide	19/24 (79%)	—	58/72 (81%)	—
B-Chain	27/30 (90%)	27/30 (90%)	75/90 (83%)	—
C-Peptide	25/35 (71%)	26/35 (74%)	76/105 (72%)	—
A-Chain	20/21 (95%)	20/21 (95%)	59/63 (94%)	57/63 (90%)
3'-Untranslated	—	—	See text	

binding[17]; nevertheless there are nucleotide changes in these regions (Fig. 3).

Protein sequence studies indicate that the C-peptide is the most variable portion of proinsulin[1-3]. Although the length of the C-peptide varies in other species[1-3], it is the same in human and rat (35 amino acids; 71% homology). The nucleotide sequence homology is 72%. The prepeptide portions of human insulin and rat insulin I are more homologous than the C-peptides (79% amino acid; 81% nucleotide). Most of the differences that occur in the prepeptides are in the carboxyl terminal portion. Indeed the first 52 bases near the amino terminus seem to be as highly conserved as the B and A chains (48/52 bases = 96%), whereas the latter 20 bases are much less conserved (10/20 bases = 50%). This suggests that this portion of the prepeptide has a function. Examination of the first 24 amino acid residues of the molecules by the sequence-predictive method of Chou and Fasman[21] shows both α-helix and β-forming potential but the former is stronger; thus two helical segments (residues −24 to −17 and −16 to −8), connected by the helix-disrupting Pro (−16), are suggested. These are the regions with the highest degree of sequence conservation. A comparison of prepeptides of other secretory proteins shows a considerable variation in the amino acid sequences, however there is always a hydrophobic region in this type of structure[22]. The strong amino acid and nucleotide sequence conservation in this region of preproinsulin would suggest some element of specificity associated with this region of the molecule. It seems unlikely that the conserved structure is concerned with the cleavage reaction since preparations of signal peptidase[23] are able to cleave molecules with different prepeptide sequences. The relatively large area of homology in the prepeptide and the possible secondary structure are more suggestive of interaction with a receptor site. Receptor-mediated endocytosis has been implicated in the specific internalisation of a number of proteins from outside the cell (see ref. 24 for refs). A receptor-mediated mechanism may also be operative at the site of internalisation of the peptides in the endoplasmic reticulum. The lack of specificity seen in the sequestration of a number of secretory proteins in pancreatic microsomes[7,25,26] could be due to a reduced degree of specificity of the receptors in this system. Alternatively, the true specificity may not have been apparent because the rates of internalisation of proteins were not measured in these experiments.

Outside the region of the mRNA encoding the preproinsulin peptide the sequences of human and rat insulin I diverge rapidly. There is only 50% homology in the short region of the 5'-untranslated sequence in the clone. The clone probably lacks about 50 bases at the 5' end that are present in the mature mRNA. The 3'-untranslated region of human preproinsulin cDNA is 21 residues longer than the cognate region of rat preproinsulin I. Despite the difference in length and the sequence divergence of this region, there is a 21-nucleotide segment adjacent to the poly(A)attachment site that is highly conserved (18/21 = 86%). These 21 nucleotides include the AAUAAA sequence found in many eukaryotic mRNAs[27]. A similar conservation of a segment of the 3'-untranslated region including the AAUAAA sequence has been observed in the mRNAs of human and rabbit β-globin[28] and in the human and rat growth hormones[18-20], although the sequences conserved are different in each case. This suggests another gene-specific element of regulation which could operate at the level of transcription, translation or polyadenylation.

The cloned human insulin cDNA will be helpful in the analysis of human genomic DNA segments which encode insulin. Moreover, it should be useful for programming the expression of human insulin in alternative hosts, such as bacteria or yeast.

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N-terminal amino acid sequencing of prolamins from wheat and related species

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The gliadins of common bread wheat (*Triticum aestivum* L.) make up the major storage protein fraction of grain endosperm and comprise a complex mixture of proteins with similar amino acid compositions and properties[1]. Two-dimensional methods of electrophoresis[2,3], in which separations are based mainly on net charge, separate gliadins of a single wheat variety into 30–46 components and there is considerable variation among the electrophoretic patterns of different wheat varieties[1]. The gliadins seem to be encoded by clusters of duplicated genes[2] and may constitute a multigene family[4]. The fact that several purified gliadin components have closely similar N-terminal amino acid sequences[5,6] led us to consider the possibility that, despite the complexity of the mixture, gliadins have sufficient homology in their N-terminal sequences to allow estimation of the homology by sequencing the unfractionated mixture. Here, we provide

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evidence for two major groups of gliadins in wheat based on N-terminal sequencing, show that similar groups of prolamins (a general term for equivalent proteins in other species) are found in related species of *Triticum* and *Aegilops* that may have contributed genomes to polyploid wheats with *ABD* genome composition, and show that only one of these groups is found in rye (*Secale cereale* L.).

Wheat and other grain samples used are listed in Table 1. The prolamins of common wheat, *Triticum monococcum*, and rye were prepared from endosperm only (flour) whereas those of other samples, of which we had only small amounts, were prepared from de-germed grain that was ground in a Wiley mill. Prolamins were prepared according to the procedure of Charbonnier[7] except that the final product was dissolved in 0.01 M acetic acid or 60% ethanol-water and dialysed thoroughly against distilled water. The small amount of precipitate that formed (5% or less of the amount dissolved) was removed by centrifugation and the clear supernatant was freeze-dried. Rye prolamins were less soluble in water—about two-thirds of the preparation precipitated—and both the precipitate and the supernatant were freeze-dried separately for use in sequencing. Yields of prolamins ranged from 3 to 8% of starting material. Electrophoretic patterns of typical preparations, which illustrate the complexity of the mixtures, are shown in Fig. 1.

Automatic sequencing[8] was carried out on a Beckman sequencer model 890B with DMAA peptide program 111374. Eight cycles were considered sufficient for our purposes. (One sample, *T. monococcum*, was sequenced only through six cycles.) Residue identification was by gas chromatography (GC) according to the procedure of Pisano *et al.*[9]. In all cases, only a few phenylthiohydantoin (PTH) amino acids (sometimes only one) were identified at each cycle of the Edman degradation and recoveries were sufficient to account for 70–80% of the protein sample when moisture contents (10%), probable sequencer yields (65% assumed[10]) and prolamin molecular weights (36,000 assumed[1]) were taken into account. Typical results are given in Table 2.

The major PTH amino acids at each cycle usually corresponded to the sequence Val-(Arg?)-Val-Pro-Val-Pro-Gln-Leu- for all species of *Triticum* and *Aegilops* except for the wheat variety 'Justin' and an accession of *Triticum urartu* (G-1876), where it was the minor sequence. This sequence corresponds to that of α-gliadins[5,6]; we shall refer to it as the α-type sequence, although Bietz *et al.*[6] found this sequence also in a β-gliadin and a γ-gliadin (γ_1-gliadin). Arg and His were not determined by our GC method. We assume Arg to be an important amino acid at position 2 by analogy with the α-gliadin sequence[5,6] and identified it at cycle 2 in two samples by liquid chromatography.

A second sequence corresponding to Asn-(Met/Ile)-Gln-Val-(Val/Asp)-Pro-Gln-Gly- was present in all the samples of *Triticum* and *Aegilops*. This sequence is similar to the γ_2- and γ_3-gliadin sequences described by Bietz *et al.*[6] (Table 2); we shall refer to it as the γ-type sequence. It was the minor sequence in most samples except for 'Justin' and *T. urartu* G-1876, where it was present in amounts about equal to (or greater than) the amount of α-type sequence. *T. urartu* G-1876, however, had only a trace amount of Asn at cycle 1; the major amino acid was Val (40 nm) followed by Ala (17 nm). We usually found both Met and Ile at cycle 2, but our sample of *T. monococcum* showed only Ile at cycle 2 whereas our sample of *Aegilops speltoides* showed only Met at cycle 2.

Table 1 Ratio of α-type to γ-type sequence from cycle 4

Species and genome designations	α/γ Ratio
T. aestivum L. em. Thell., 'Scout 66' (*ABD*)	1.6
T. aestivum L. em. Thell., 'Justin' (*ABD*)	0.9
T. dicoccoides Körn., 64–1148 (*AB*)	1.1
T. dicoccum Schubl., Khapli emmer, 63–2356 (*AB*)	1.3
T. boeoticum Boiss. em. Schiem., G–2512 (*A*?)	2.2
T. monococcum L., Univ. Manitoba (*A*?)	1.3
T. urartu Tum., G–3151 (*A*?, *B*?)	1.1
T. urartu Tum., G–1876 (*A*?, *B*?)	0.7
Ae. speltoides Tausch, U. C., Davis (*B*?)	1.4
Ae. squarrosa L., 0–623 (*D*)	1.9
Ae squarrosa L., 64–1154 (*D*)	1.9
Secale cereale L., 'Frontier'	—

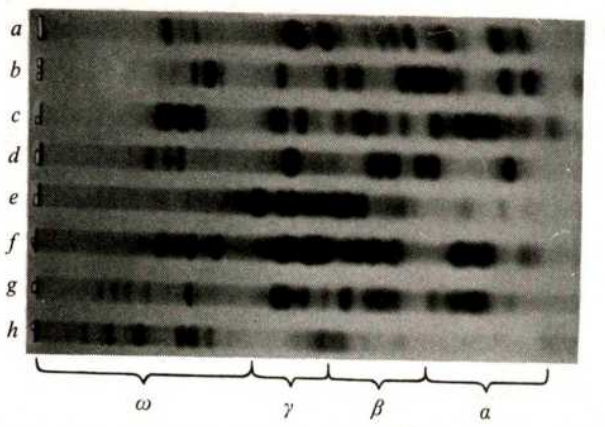


Fig. 1 Polyacrylamide gel electrophoresis[16] of prolamins from different species, aluminium lactate buffer, *p*H 3.2, migration from left (+) to right (−): *a*, *T. monococcum*; *b*, *T. boeoticum* (G-2512); *c*, *T. urartu* (G-3151); *d*, *Ae. squarrosa* (0-623); *e*, *Ae. speltoides*; *f*, *T. dicoccoides* (64-1148); *g*, *T. aestivum* ('Scout 66'); *h*, *S. cereale* ('Frontier'). The α-, β-, γ- and ω-regions correspond to the usual assignments of electrophoretic mobilities for gliadin patterns of common wheats[1].

On the basis of N-terminal sequences, our results indicate two major homologous groups of prolamins in the species of *Triticum* and *Aegilops* we examined. Because these species were fairly representative of the two genera, this may be true in general. Based on sequencer yields, we estimate that these two groups account for about 70–80% of our prolamin preparations. A fairly clear difference between the α-type and γ-type sequences occurs at residue 4, where Pro is found in the former and Val or Gln in the latter (Table 2). Pro and Val were the only two amino acids we identified at cycle 4 for all species of *Triticum* and *Aegilops* with the exception of one accession of *T. urartu* (G-1876), which also had a small amount of Leu at this position. The ratio of Pro to Val at cycle 4 may provide a measure of the relative amounts of the two groups of proteins in a sample. These ratios are listed in Table 1, and range from 0.8 to 2.2. They sometimes differed for different varieties or samples of the same species. For example, the two hexaploid bread wheats, 'Scout 66' and 'Justin', had ratios of 1.6 and 0.8, respectively. The large amount of α-type sequence in 'Scout 66' is consistent with the large amounts of α-gliadins in this variety[11]. In contrast, 'Justin' is low in α-gliadins[11]. The presence of both α-type and γ-type prolamins in species of *Triticum* and *Aegilops* indicates the close relationship of these genera, and supports the proposal of Morris and Sears[12] that *Aegilops* be incorporated into the genus *Triticum*.

Both the water-soluble and the water-insoluble prolamins from 'Frontier' rye differed from those of the other species examined in lacking the α-type sequence, although we might not have detected a sequence corresponding to about 10% or less of the mixture. The sequences of these rye prolamin preparations were not identical to one another but both were fairly close to the γ-type found in species of *Triticum*[6] and *Aegilops*. Although minor amino acids were identified at most cycles of the rye prolamin degradation, these did not correspond to known gliadin sequences. The electrophoretic patterns of rye prolamins at *p*H 3 vary somewhat according to variety[13], but they do not show a significant amount of staining in the region of mobility corresponding to α-gliadins[13]. As we have noted above, however, the α-type of sequence has been found for proteins with other electrophoretic mobilities[6], so the agreement between our sequencing results and the electrophoretic patterns may not be essential.

Table 2 Recovery of PTH amino acids for different species (nmol)

Species	Cycle of Edman degradation 1	2	3	4	5	6	7	8
T. aestivum 'Scout 66'	Val (97) Asn (37)	* Met (40) Ile (22)	Val (65) Gln (54) Leu (12)	Pro (75) Val (47)	Val (69) Asp (28)	Pro (91)	Gln (71) Ser (50)	Leu (64) Gly (37)
T. aestivum 'Justin'	Val (55) Asn (51)	* Met (42) Ile (40)	Val (50) Gln (39) Leu (32)	Pro (53) Val (63)	Val (41) Asp (24)	Pro (104)	Ser (56) Gln (20)	Leu (46) Gly (42)
T. dicoccoides 64–1148	Val (64) Asn (33)	* Met (33) Ile (29)	Val (63) Gln (29) Leu (21) Phe (5)	Pro (76) Val (72)	Val (68) Asp (16)	Pro (109)	Ser (70) Gln (25)	Leu (59) Gly (41)
Ae. squarrosa 64–1154	Val (119) Asn (26) Ala (12)	* Ile (39) Met (17)	Val (91) Gln (25)	Pro (94) Val (47)	Val (53) Asp (9)	Pro (99)	Gln (43)	Leu (39) Gly (10)
S. cereale 'Frontier' (soluble fraction)	Asn (59) Ala (24) Leu (20)	Met (97) Gln (87)	Gln (95) Leu (78)	Val (35) Asn (15)	Pro (48) Gly (41) Asn (15)	Pro (59) Ser (57)	Ser (73) Gln (14)	Gly (53) Gln (43)
α-gliadin[5,6]	Val	Arg	Val	Pro	Val	Pro	Gln	Leu
γ_2-gliadin[6]	Asn Pro	Ile	Gly Gln	Val	Asp Val Gln	Pro Gln	Trp	Gly Leu
γ_3-gliadin[6]	Asn Pro	Met	Gly Gln	Val Gln	Asp Val	Pro Gln	Trp Gln	Gly

Recoveries are reported on a basis of 10-mg samples.
* Arg is likely to be an important residue at position 2, but Arg and His were not determined by our GC method: in addition, the quantitation of Asn and Gln was less accurate than that of the other amino acids.

The presence of only the γ-type sequence in our sample of rye prolamin may indicate that rye is closer evolutionarily than *Triticum* and *Aegilops* to the common ancestor that gave rise to the sub-tribe Triticinae[14], which includes both wheat and rye, within the family Gramineae. Differentiation may have included the development and amplification of genes coding for α-type prolamins in *Triticum* and *Aegilops*. This speculation is based on results for only one variety of rye; further work on additional varieties and types is needed to allow its generalisation to the species as a whole.

The great homology among the mixtures of prolamins we have studied enables considerable sequencing information to be obtained from them without isolating single protein components. This has also been demonstrated[15] for the zein proteins, the prolamins of maize (*Zea mays*). This homology is notable in consideration of the complexity of the mixtures. The prolamins of species of *Triticum* and *Aegilops* consist of many components that may differ in molecular weight as well as in charge; prolamin preparations from various species of these genera were resolved into at least four (usually more) bands by SDS electrophoresis (E. C. Cole, J. G. Fullington and D.D.K., unpublished results). Small differences in amino acid composition have been found for gliadin components that differ in electrophoretic mobility (aluminium lactate buffer, *p*H 3.2)[1], and gliadin proteins corresponding to particular bands of the *p*H 3.2 patterns exhibited simple inheritance in crosses between wheat varieties that differed in their electrophoretic patterns[2]. Regardless of whether the complexity of gliadins and other prolamin mixtures results from multiple genes, post-translational modifications or some other mechanism, the high degree of homology we found for their N-terminal sequences suggests that this region has some important function and thus tends to be conserved.

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Proposed structure of the anthramycin–DNA adduct

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Anthramycin, an antitumour antibiotic produced by *Streptomyces refuineus*[1], belongs to the pyrrolo(1,4)benzodiazepine antibiotic group[2]. Antibiotics within this group are potent inhibitors of nucleic acid synthesis because of their ability to form a labile covalent adduct with DNA[2–6]. The reaction of anthramycin with DNA is unusual in that the rate of adduct formation is slow[2,4,5], shows an absolute specificity for deoxyguanosine in a double-stranded template[4], and is only stable as long as the secondary structure of DNA is retained[6]. Experiments using confluent non-dividing human cell lines have demonstrated that while repair-proficient cells are able to remove up to 84% of the adduct within 72 h, xeroderma pigmentosum cells in complementation group A were only able to remove 49% during the same incubation period[10]. We propose here a Corey, Pauling and Koltun (CPK) molecular model for the anthramycin–DNA adduct in which anthramycin is attached through the 2-amino group of guanine and lies hidden in the narrow groove. Experimental data supporting this model are provided. The model is based on information about the probable covalent binding sites on anthramycin and guanine together with the known crystal structure of anthramyin[7].

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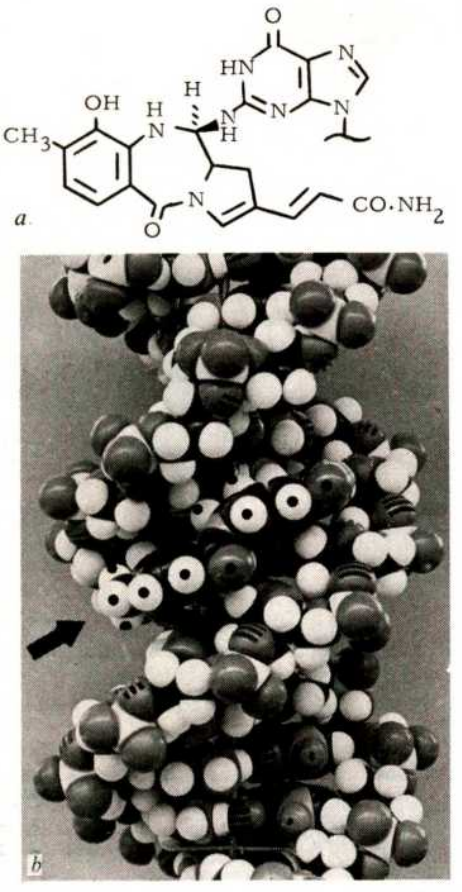


Fig. 1 Structure (*a*) and CPK space filling model (*b*) of the anthramycin–DNA adduct in which anthramycin is attached through C-11 to the N-2 group of guanine.

Structure activity relationships for anthramycin and related compounds provide compelling evidence that the carbinolamine is the reactive group on anthramycin[2,4,5,14]. X-ray diffraction studies on crystals of anthramycin show that the molecule is twisted 40 to 50° from one end to the other along the long axis and thus might fit into one of the grooves of DNA[7]. From studies using synthetic polydeoxynucleotides, guanine is apparently the reactive base on DNA[4]. Of the possible positions for alkylation by anthramycin, N-7, N-3, and C-8 have been eliminated because of: (1) the results of an alkylation assay using [8-^{3}H]guanine-labelled DNA; (2) absence of strand breakage *in vitro* using SV40 DNA; and (3) the lack of increased susceptibility to depurination of the anthramycin modified [8-^{3}H]guanine-labelled DNA[8]. The unreactivity of polydI:dC towards anthramycin, while polydG:dC binds the drug at least as well as native DNA[4], strongly suggests that the N-2 position on guanine is directly involved in adduct formation. Model building using a CPK molecular model of DNA and anthramycin in the published crystal conformation demonstrated quite dramatically that while O-6 was inaccessible to adduct formation without bond cleavage or serious distortion of DNA, adduct formation through N-2 of guanine produced a remarkably snug fit. The anthramycin molecule which is attached through C-11 to N-2 of guanine by an aminal linkage fits within the narrow groove of DNA (Fig. 1). The predictions which result from this model and the experimental data substantiating our model are presented below.

At saturation binding in DNA, where 25% of the bases are guanine and the drug molecule covers five base pairs, the maximum binding ratio would be 1:10. However, as the distribution of guanine residues is not uniform the frequency will be somewhat lower, and the experimentally determined ratio of 1:12. 8 (ref. 2) is probably close to the theoretical value.

Binding of anthramycin should not cause unwinding or lengthening of DNA as the drug binds in a non-intercalative manner within the narrow groove. Viscosity and sedimentation measurements have shown that the DNA molecule is stiffened but not lengthened on anthramycin binding[9]. Experiments using SV40 DNA, predominantly in the supercoiled form, have shown no unwinding at an antibiotic to base ratio of 1:300.

Our model shows that the angle of the chromophore of anthramycin is inclined at 45° relative to the helix axis. This compared very well with the reported value of 36° as determined by electric dichromism measurement[9].

The drug is held to DNA by an animal linkage plus secondary stabilising forces, chiefly between the phenolic group at C-9 of anthramycin and the 2-keto group of cytosine in the same base pair. As the animal linkage is inherently unstable, conditions which remove the secondary stabilising interaction should lead to release of anthramycin from the adduct. The predictions are mimicked in practice as denaturation of the DNA by acid *p*H (ref. 5), heat and enzymatic degradation[6], lead to rapid release of free drug from the DNA adduct.

In the proposed model, anthramycin fits into the narrow groove of DNA without distortion or protrusion outside of the helix. To probe the modified DNA for distortion or protrusion of the adduct outside the helix, S_1 nuclease digestions and benzoylated napthalated DEAE (BND)-cellulose chromatography on the adducts were carried out. The results (Fig. 2, Table 1) show that S_1 nuclease was unable to reveal any regions of distortion in the drug-modified DNA and, in fact, release of nucleotides was inhibited with increasing amounts of drug modification. The BND-cellulose chromatography results demonstrated lack of single strandedness of DNA induced by anthramycin as well as binding of anthramycin to the resin which would be anticipated if the drug protruded outside the DNA helix.

The orientation of anthramycin in the narrow groove allows interactions between the phenolic group at C-9 of anthramycin and the 2-keto group of cytosine. Titration[4] and CD measurements[9] have shown that in the adduct, the phenolic hydroxy proton is no longer available for titration, which is in accord with

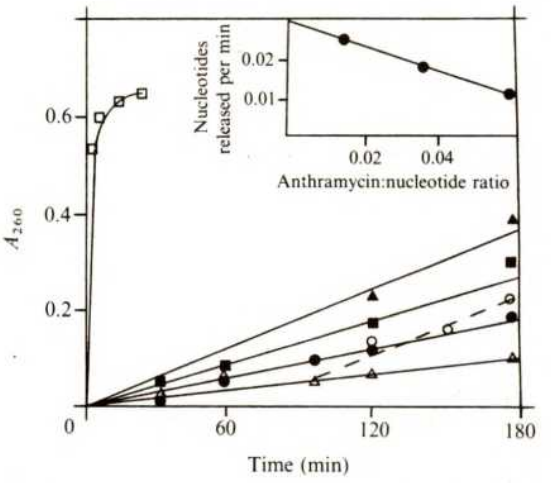


Fig. 2 Time course hydrolysis of calf thymus DNA during digestion with S_1 nuclease in the presence of increasing amounts of bound anthramycin. Assays were carried out essentially as described by Vogt[13]. Calf thymus DNA in 0.1 M SSC was diluted into reaction buffer to produce a final concentration of 0.03M Na acetate *p*H 4.6, 0.025 M NaCl and 0.005 M $ZnSO_4$. The reactions were carried out at 37 °C. Aliquots were removed at various times and transferred into 0.4 ml cold 10% perchloric acid. The samples were centrifuged (10,000*g* for 15 min) and the A_{260} of the supernatant was measured to determine the release of nucleotides by the enzyme. The anthramycin:nucleotide ratio was 0(▲); 0.015 (■); 0.037 (●); 0.063 (△); DNA concentration 150 μg ml^{-1}, enzyme concentration 2×10^3 U ml^{-1}. Heat denatured DNA (□). At 90 min unmodified calf thymus DNA was added to an enzyme incubation containing drug-modified calf thymus DNA (○). Inset, dependence of the nucleotides released per min against anthramycin:nucleotide ratio.

Table 1 Estimation of the single strandedness and protrusion of anthramycin molecule external to DNA found in the anthramycin-DNA adduct using BND-cellulose chromatography

Anthramycin: nucleotide ratio	% Adherence* DNA (A_{260})	^{3}H-Anthramycin (c.p.m.)
0	6	–
1:1134	4†	12†
1:144	3	12
1:34	2	10

Chromatography on BND-cellulose in which step elution using 1 M NET (1.0 M NaCl–10^{-4} M, EDTA-0.01 M Tris HCl, *p*H 7.5) eluate and a 50:50 1 M NET: formamide mixture can be used to estimate the double-stranded DNA (eluted with 1 M NET) against DNA containing single-stranded regions[11] or protruding aromatic moieties[12] in a sample of DNA. To obtain DNA without single-stranded breaks or regions, calf thymus DNA (Sigma) was pre-chromatographed on BND-cellulose. The 1 M NET eluate (double-stranded DNA) was dialysed against 0.1 SSC, bound to varying concentrations of [15-^{3}H]anthramycin (4.0 μCi μmol^{-1})[5], and again dialysed against 0.1 SSC to remove any unbound antibiotic. Control DNA was also dialysed. The DNA–anthramycin adduct (140–220 μg DNA) was then applied to BND-cellulose columns (1 ml column volume) and eluted successively with 10 ml of 0.3 M NET (0.3 M NaCl–10^{-4}, EDTA-0.01 M Tris HCl, *p*H 7.5), 10 ml of 1.0 M NET and 10 ml of 50% formamide in 1.0 M NET. 2.0 ml fractions were collected. Radioactivity was determined by adding 1.0-ml aliquots to 10 ml of Aquasol (NEN) and DNA by optical density measurement at 260 nm. Optical density measurements of the formamide: 1.0 M NET fractions were corrected for background absorption using a control column without adding DNA.

* The % adherence represents the fraction of absorbance at 260 nm and the fraction of radioactivity eluted with 50% formamide in 1 M NET out of the total amounts eluted with 1 M NET and 50% formamide in 1 M NET.

† The larger values for the apparent percentage adherence found in the radioactivity determinations are most probably due to a small amount of release of drug from the DNA during manipulation before and during chromatography.

our model in which hydrogen bonding between the phenolic group and the 2-keto of cytosine is possible. Our model also accounts for the tighter binding of sibiromycin to DNA than anthramycin as the amino sugar attached at C-7 of sibiromycin binds externally to the deoxyribsose phosphate backbone of the helix.

As the anthramycin–DNA adduct is well concealed within the narrow groove of DNA it might be anticipated that excision repair would be a slow process due to the lack of distortion or protrusion which would normally lead to ready recognition of the drug-modified DNA. In practice the drug is only excised relatively slowly over a 48-h period in confluent normal non-dividing human cells[10].

This proposed model for the anthramycin-DNA adduct explains fully both the *in vitro* and *in vivo* data so far obtained. The proposed type of binding to DNA in which the drug molecule is attached by a labile covalent adduct and resides inside the narrow groove seems to be unique to the pyrrolo(1,4)benzodiazepine antibiotics and perhaps explains some of the unusual biological effects of this group of agents. Further experiments using stable isotopically labelled anthramycin and synthetic polydeoxynucleotides should vigorously establish the points of covalent linkage.

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Energy uptake in the first step of visual excitation

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Perception of light by the retina starts with the absorption of a photon by 11-*cis* retinal, which is covalently incorporated into the membrane-bound protein, rhodopsin[1]. The initial result of photon capture is the very rapid formation of a red-shifted species, bathorhodopsin[2–4] (also known as prelumirhodopsin), which is (meta-)stable at liquid nitrogen temperature but which decomposes at higher temperatures, in the dark, through a series of intermediate stages, resulting in the release of all-*trans* retinal from the apoprotein, opsin. Bathorhodopsin formation is the only photochemical step in the overall reaction and, therefore, merits investigation. Several models for the process have been proposed, and have been critically reviewed[5–8], although no consensus yet exists as to the nature or mechanism of formation of the batho intermediate. I report here on the first direct measurement of photon energy uptake during bathorhodopsin formation from bovine rhodopsin, and on its possible significance.

We have previously described in detail the technique of photocalorimetry[9] and its application to the determination of the energetics of the later stages in the photolysis of rhodopsin[9] and isorhodopsin[10]. The present study required the construction of a calorimeter suitable for use at very low temperatures (Fig. 1). This instrument is based on the isothermal heat conduction principle[11] and allows direct measurement of the total heat energy dissipated in a sample during monochromatic illumination. In the differential mode, the energy of the exciting light may be subtracted automatically, yielding directly the photochemically-induced uptake or release of energy in the sample. The present instrument has a sensitivity of about 0.1 μV μW^{-1} at 298 K, falling to about 0.02 μV μW^{-1} at 77 K due to the intrinsic thermal properties of the thermoelectric sensors. Bovine rod outer segments, prepared by standard sucrose gradient techniques[12] were suspended in either aqueous or neutral detergent (Ammonyx-LO)–glycerol mixtures and irradiated in the photocalorimeter (in liquid nitrogen) at 450 or 485 nm. Samples enriched in bathorhodopsin were generated *in situ* by irradiation at 485 nm and photoreversal was induced by irradiation at 560 nm (ref. 3). Extents of photoreaction were determined by the change in absorbance at 500 nm ($\varepsilon = 41{,}000$) of samples warmed to room temperature and dispersed in 2% (w/v) cetyltrimethylammonium bromide (CTAB), 0.1 M phosphate *p*H 7.0, 10 mM hydroxylamine. Typically, 10–20 nmol of rhodopsin were bleached in any one experiment.

Illumination of rhodopsin at 77 K gives rise to an unambiguous uptake of energy in parallel with bathorhodopsin formation (Fig. 2*a*), and this stored energy is released (Fig. 2*b*) on photoreversal to rhodopsin. (Formation of some 9-*cis* pigment, isorhodopsin, is to be expected during photoreversal[3] but, as its energy is only about 5 kcal mol^{-1} higher than rhodopsin[10] this has no major effect on the observations.) No net heat effects were observed in control experiments with totally bleached rhodopsin samples. The combination of a series of

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determinations yields:

$$\text{rhodopsin} \xrightarrow[77\,K]{h\nu} \text{bathorhodopsin}$$

$$\Delta H = +34.7\ (\pm 2.2)\quad \text{kcal mol}^{-1}$$

Within experimental error identical results are obtained for irradiation at two different wavelengths (450 and 485 nm), and for both detergent-solubilised rhodopsin and outer segment suspensions. The latter observation suggests that, at this level, the precise molecular environment of the rhodopsin molecule has little influence on the energetics of the primary process.

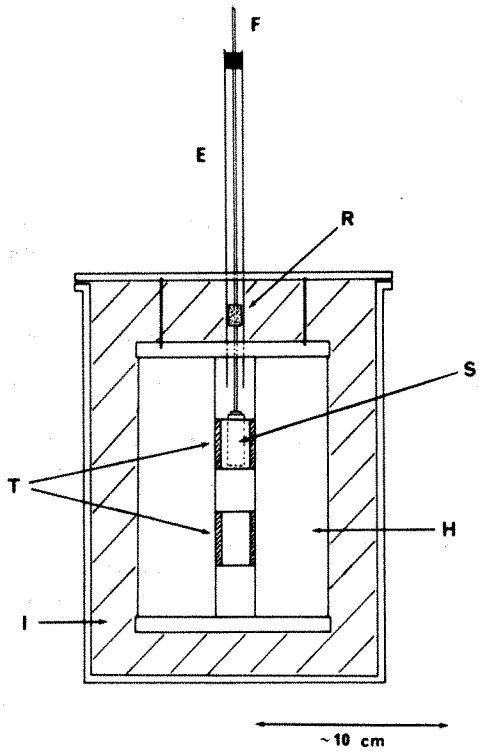


Fig. 1 Low temperature photocalorimeter. The sample (up to 2.5 ml) is enclosed in a cylindrical gold-plated, stainless-steel cell (S) fitted with flexible fibre-optics light guides (F) for illumination. A coaxial thermal radiation shield (R) minimises thermal leakage down the sample entry tube (E). The thermoelectric sensors (T) are solid-state Peltier cooling devices (Borg-Warner 950-71), the output voltage of which is proportioned to the rate of heat flow between the sample block and the heat sink (H). The second (lower) set of sensors, with dummy sample block, is connected in opposition to reduce the effects of thermal fluctuations in the heat sink. A second sample cell (not shown) is mounted behind and parallel to the first, such that differential measurements may be made. Small heater coils are permanently mounted in the sample block for electrical calibration. Insulation (I) is with expanded polystyrene foam. The entire unit was suspended in a liquid nitrogen Dewar vessel, with the upper end of E contained in a N_2-flushed dry box to eliminate condensation during sample loading and removal. Light source was a 150 W xenon arc and grating monochromator, with appropriate optics for coupling to the fibre-optics light guides. Sensor voltages were monitored with a Keithley 150B μV ammeter, averaged and integrated over 1-min intervals, and presented digitally. The equipment was installed in a temperature-controlled darkroom.

Thus, the ground-state energy of bathorhodopsin is about 35 kcal (145 kJ, 1.50 eV) higher than rhodopsin. It had been anticipated that the primary reaction should be endothermic, as the thermal instability of bathorhodopsin implies an elevated thermodynamic free energy[5,9,13], and entropy contributions are not expected to be significant at low temperatures[7]. The magnitude of the energy storage is, however, much higher than theoretical models have suggested[14,15], amounting to more than 60% of the absorbed photon energy at the 500-nm absorbance maximum of bovine rhodopsin. It is also significantly higher than the measured barrier of ~25 kcal for thermal isomerisation about the conjugated double bonds of free retinal[16]. If, as seems most probable on evidence so far[7], bathorhodopsin formation involves at least partial rotation about the retinal 11–12 double bond, then interactions in the retinyl binding site of the protein have a dominating influence on the process, forcing dramatic changes in the conformational potential energy map of the chromophore.

It has been argued that rhodopsin and bathorhodopsin share a common, barrierless, excited-state energy surface[5–7]. Knowing the ground-state energy of bathorhodopsin, together with spectroscopic and other data, it is possible to begin to reconstruct a more quantitative picture of the potential energy surfaces for the photoreaction (Fig. 3). The interesting possibility then arises that the energy surfaces for the ground and excited states might actually overlap in some region of reaction-space. This conjecture arises from the following considerations: (1) the energy of bathorhodopsin is high (35 kcal above rhodopsin); (2) thermal decay of bathorhodopsin to the next intermediate, lumirhodopsin, involves an activation barrier of about 10 kcal (ref. 17) and, as thermal regeneration of rhodopsin from bathorhodopsin has not been observed, this suggests a ground-state barrier in excess of 45 kcal between rhodopsin and bathorhodopsin; and (3) the excited-state surface must have a minimum below 57 kcal (≡500 nm) in order to guide the system towards bathorhodopsin[5]. A hint to the possible intersection or overlap of the energy surfaces comes from the classic experiments of St George[18], which showed that at long wavelengths rhodopsin photolysis becomes temperature dependent, and that

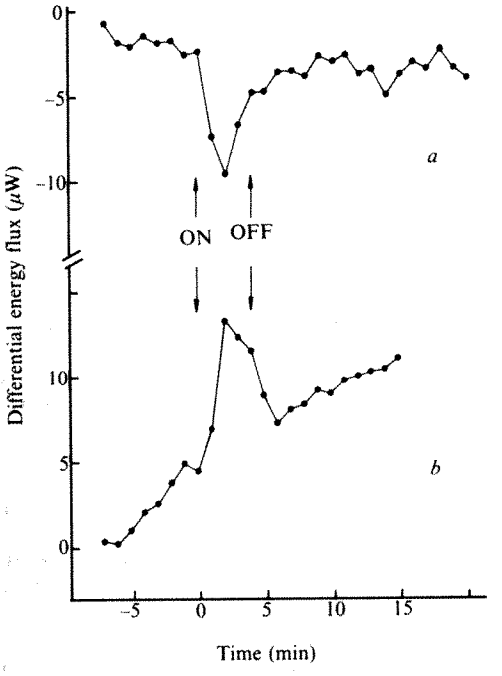


Fig. 2 Differential thermal response on irradiation of rhodopsin and bathorhodopsin in the photocalorimeter at 77 K. The sample consists of bovine rod outer segments dispersed in a mixture of glycerol (67%), Ammonyx-LO (2.5%), 10 mM phosphate buffer pH 7.0. *a*, Rhodopsin → bathorhodopsin: 4 min irradiation at 485 nm resulting in a net heat uptake. About 15 nmol of rhodopsin (~20% of the sample) were bleached in this experiment. *b*, Bathorhodopsin → rhodopsin: the above sample was irradiated continuously at 485 nm to produce a steady-state mixture of bathorhodopsin, rhodopsin and isorhodopsin. Subsequent 4 min irradiation at 560 nm (shown) demonstrates the exothermic photoreversal of bathorhodopsin to rhodopsin. (Baseline drift in these experiments is caused by slow temperature variation in the calorimeter, estimated to be less than 5×10^{-6} °C min^{-1}).

thermal and electromagnetic energy can combine to bleach rhodopsin. The critical wavelength for onset of this phenomenon is 590 nm, suggesting a possible intersection of the energy surfaces at about 48 kcal. Figure 3 has been drawn with this energy in mind.

Given this topology, the response of rhodopsin to photon absorption might be as follows: vertical (Franck–Condon) excitation to a high vibrational level of the excited state followed by rapid vibrational relaxtion to lower levels and efficient crossover to isoenergetic states of the ground-state surface in the intersecting region. Further relaxation to the ground-state of bathorhodopsin, or back to rhodopsin, would depend on the dynamics of the situation but, experimentally, bathorhodopsin is favoured in the ratio of about 2:1 (refs 5, 6).

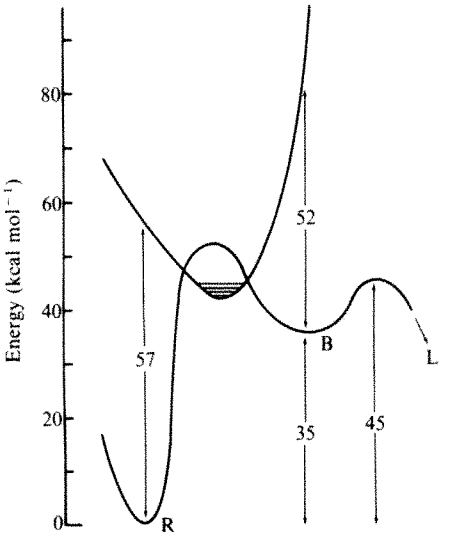


Fig. 3 Two dimensional projection of the possible ground- and excited-state potential energy surfaces for the primary step in rhodopsin photolysis. The ground-state energy of bathorhodopsin (B) with respect to rhodopsin (R) is from direct measurement. Vertical excitation energies to the electronically excited state are from absorbance maxima (500 and 548 nm for R and B, respectively). The B → L (lumirhodopsin) barrier is from kinetic data[17], and suggests a lower limit to the R → B barrier in the ground state. The possible overlap (in projection) of the two surfaces is, probably, exaggerated for clarity. The hatched region indicates possible trapping in lower vibrational levels of the excited-state energy minimum at low temperatures. The nature of the reaction coordinate needs further clarification, but probably includes the retinal 11–12 torsion angle[5–7] as well as other chromophore and protein parameters.

One interesting consequence of energy surface overlap is that at very low temperatures excited molecules might get trapped in vibrational states of lower energy than the ground state of the same conformation (Fig. 3), leading to an energy barrier and a temperature dependence in the rate of bathorhodopsin formation from the excited state, perhaps as observed in picosecond studies[19]. However, even at the very lowest temperatures, some crossing over to the ground-state surface might be expected before complete thermal relaxation after excitation, and non-Arrhenius kinetic behaviour might be seen, possibly without the need to invoke quantum mechanical tunnelling processes[19]. Deuterium isotope effects[19] would arise in this situation from changes in the vibrational energy levels of modes involving exchangeable hydrogens on the protein and chromophore.

Although we have yet to understand the process in molecular terms, it seems clear that protein–chromophore interactions in rhodopsin have evolved to provide a smooth and efficient pathway for the inital photochemical step in photoreception, whilst maintaining sufficient stored energy to drive the subsequent processes which give rise to the visual stimulus.

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Evidence for nitrogen fixation (*nif*) genes on indigenous *Rhizobium* plasmids

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The distinguishing characteristic of bacteria that belong to the genus *Rhizobium* is their ability to induce nodules and fix nitrogen in the roots of leguminous plants—hence their importance in agriculture. The demonstration of nitrogen-fixation by rhizobia in the absence of host plant material showed that the genes for nitrogenase synthesis and function reside in the bacterium[1–3]. The presence of large plasmids (molecular weight (MW) 90–350 × 10⁶) in many *Rhizobium* strains is now well established[4–6] and earlier genetic evidence suggested that at least some of the genes required for symbiosis were plasmid-linked[7,8]. More recently, Johnston *et al.*[9] found evidence that genes required for nodulation were carried on a plasmid in *Rhizobium leguminosarum*. We now report hybridisation between restriction fragments of plasmid DNA from *R. leguminosarum* and DNA encoding part of the structural genes for *Klebsiella pneumoniae* nitrogenase. Our results provide evidence that at least some of the *Rhizobium nif* genes are carried on plasmids indigenous to the strains examined.

Overlapping sequences of *K. pneumoniae nif* DNA which together encode the 15 known *nif* genes have been cloned on small amplifiable plasmids (refs 10 and 11 and unpublished results of M. C. Cannon and F.C.C.). The *nif* genes carried on plasmids pSA30 and pCM1 are shown in Fig. 1. The three structural genes for nitrogenase, *nif*KDH and part of *nif*E are encoded by a DNA fragment (MW 4.2 × 10⁶) cloned in pSA30. An overlapping fragment carrying *nif*Q–E and part of *nif*K is

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cloned in pCM1 (refs 11, 12). These plasmids were used as radioactive probes to check for the presence of *nif* DNA sequences in *Rhizobium* plasmids. Homology between *nif* DNA from *K. pneumoniae* and *Rhizobium* was expected because: (1) all characterised nitrogenase proteins show a high degree of similarity in their physicochemical properties; (2) component proteins of nitrogenase from different sources form active heterologous enzyme complexes in many of the cases examined; (3) statistical analyses of the amino acid composition of different nitrogenases predicted a high degree of sequence homology[13].

Plasmid DNA from three strains of *R. leguminosarum* was examined. Strain LPR1705, which is a derivative of the wild-type strain A171, nodulates (Inf^+) *Pisum sativum* effectively (Eff^+)[5]. LPR180, which is Inf^-, was isolated as a non-gummy variant of LPR1705 (ref. 5). JB897 (*phe, trp, str*), which is a derivative of *R. leguminosarum* strain 300 (ref. 14), nodulates peas effectively. The MWs reported[5,6] for at least three plasmids detected in strains LPR1705 and JB897 fall within the ranges, $100–110\times10^6$, $165–200\times10^6$ and $200–240\times10^6$. The plasmid of MW $100–110\times20^6$ was absent in strain LPR180.

The procedure used to investigate *Rhizobium* plasmids for the presence of sequences homologous to *Klebsiella nif* DNA involved: (1) quantitative purification of *Rhizobium* plasmid DNA (refs 15 and 16 and unpublished result of M.P.N. and R.K.P.); (2) digestion of the DNA with restriction endonuclease *Eco*RI; (3) separation of the DNA restriction fragments by agarose gel electrophoresis; (4) denaturation of the DNA fragments in the gel; (5) transfer of the single-stranded DNA bands to nitrocellulose sheets using the Southern technique[17]; (6) hybridisation with probes of *Klebsiella nif* or control DNA labelled with ^{32}P *in vitro*[16,18]. Figure 2 shows the hybridisation results obtained with the nitrogenase structural gene probe, pSA30. Two DNA bands (MWs 1.67 and 1.33×10^6) from each of strains LPR1705 and LPR180 hybridised to the probe. The hybridisation was weaker with the smaller band from both strains. Three bands (MWs 5.8, 1.67 and 1.4×10^6) from strain JB897 hybridised, one more strongly than the others to pSA30. When pACYC184, the vector for the *nif* genes of pSA30 (Fig. 1), was used as a probe, hybridisation to the above bands was not observed, indicating that the hybridisation of these bands with pSA30 was *nif*-specific. A third band (MW 3.41×10^6) from LPR1705 which hybridised weakly to pSA30 also hybridised to the pACYC184 probe and therefore is probably not *nif* DNA. Further evidence which suggested that the hybridisation was *nif*-specific was obtained from a control experiment in which hybridisation was not observed between pSA30 probe DNA and *Eco*RI digestion fragments of large plasmid DNA from *Agrobacterium tumefaciens* strain Ach5 (ref. 19), a non-nitrogen-fixing member of the Rhizobiaceae. Plasmid pCM1, a probe which carries part of the nitrogenase structural gene, *nif*K and 11 of the 12 other identified *nif* genes, did not hybridise. This suggests that detectable homology between *Rhizobium* and *Klebsiella nif* DNA is confined to part of the nitrogenase structural genes.

We conclude from these results that the *Rhizobium* plasmid DNA examined contains sequences which are homologous to part of the structural genes for nitrogenase from *K. pneumoniae*. This is strong evidence that at least some of the structural genes for *R. leguminosarum* nitrogenase are plasmid borne. These genes cannot be on the plasmid of MW $100–110\times10^6$ in LPR1705 because this plasmid is absent in the Inf^- derivative strain LPR180 which gave an identical hybridisation pattern.

We have also examined plasmid DNA from *Rhizobium meliloti* L5-30 for the presence of sequences homologous to *K. pneumoniae nif* genes. Intensive investigations have revealed only one plasmid of MW 90×10^6 in this strain[5,6]. Our preliminary results show that two *Eco*RI fragments of DNA (MWs 1.82 and 1.6×10^6) hybridised to the *nif* DNA of pSA30 but not of pCM1. Ruvkun and Ausubel (personal communication) have observed homology between *Rhizobium trifolii* plasmid DNA and the *nif* genes on pSA30, which is consistent with the transfer of *nif* genes from *R. trifolii* to *K. aerogenes* reported by Dunican and Tierney[8]. These results suggest that the presence of *nif* genes on indigenous plasmids is widespread among *Rhizobium* species. These plasmids can now be investigated for self-transmissibility using the method reported by Beringer *et al.*[20] for inserting the transposon Tn*5* into *Rhizobium* plasmids.

Our results demonstrate that a combined molecular and genetical analysis of *Rhizobium nif* genes is now possible. Probes of *Klebsiella nif* DNA encoding homologous parts of the

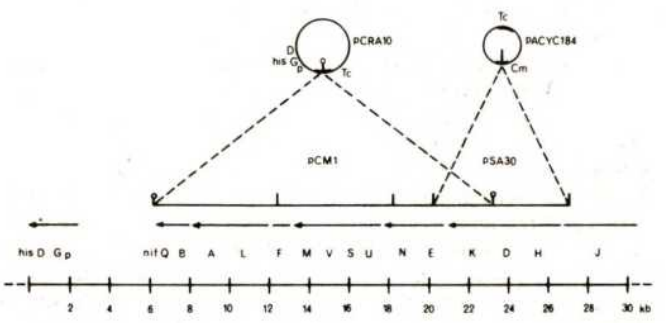


Fig. 1 *Nif* DNA restriction fragments cloned in pCM1 and pSA30 (ref. 9). Symbols for restriction endonuclease sites are: *Hin*dIII, ↥ and *Eco*RI, ⊥. The *his–nif* DNA region and the cloning vectors, pCRA10 and pACYC184, are drawn to the same scale. The arrows indicate *nif* and part of the *his* operons and their direction of transcription—*nif*F and *nif*J are monocistronic operons. The map of *nif* genes and operon structure are from MacNeil *et al.*[21], Riedel *et al.*[12] and Merrick *et al.* (in preparation). Tc and Cm are symbols for tetracycline and chloramphenicol resistance genes.

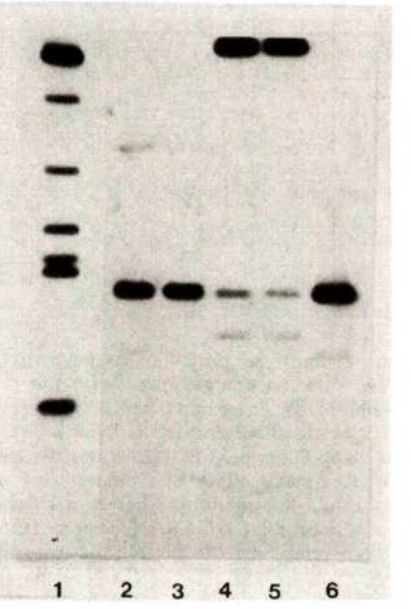


Fig. 2 Autoradiogram of ^{32}P-labelled pSA30 DNA hybridised to a Southern transfer of control DNA and of *Eco*RI-digested plasmid DNA from *R. leguminosarum* strains. *Eco*RI-generated fragments of *Rhizobium* plasmid DNA, separated by agrose gel electrophoresis were transferred to a nitrocellulose sheet and hybridised to the radioactive probe by a modification[16] of the Southern[17] method. pSA30 probe was labelled *in vitro* to a specific activity of $1–2\times10^8$ d.p.m. per μg (ref. 16). DNAs on the nitrocellulose sheet were: lane 1, MW standards (5.1, 4.15, 2.65, 2.2, 1.9, 1.69, 0.96×10^6) obtained by combining 100 pg of pSA30 DNA digested with *Eco*RI, 200 pg of pSA30 DNA digested with *Eco*RI + *Hin*dIII, and 400 pg of pCM1 DNA digested with *Eco*RI; lanes 2–6, plasmid DNA (2 μg) for strains LPR1705 (lanes 2 and 3), JB897 (lanes 4 and 5) and LPR180 (lane 6).

structural genes for nitrogenase could be used to clone *Rhizobium nif* genes, and all the *nif* mutants of *K. pneumoniae* which have been characterised in the past decade are potential hosts for examining such cloned material. These probes could also be used to determine the locations of *nif*::Tn insertion mutations and so to construct a physical map of the *Rhizobium* nitrogenase genes analogous to that of *K. pneumoniae*[12].

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Inhibition of spinach glyceraldehyde-3-phosphate dehydrogenases by pentalenolactone

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The antibiotic pentalenolactone, isolated from the fermentation broth of various species of *Streptomyces*[1–3], has three different forms depending on culture conditions and purification procedure: E (epoxide; Fig. 1), D (diol) and C (chlorhydrine). The latter two derive from E by cleavage of the epoxide ring with insertion of the elements of water and HCl, respectively. As *in vitro* studies have shown[6], pentalenolactone inhibits glycolysis by inhibiting specifically glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), but no other enzyme was known to be affected. The inhibition is irreversible and is similar whether the enzyme comes from *Escherichia coli*, yeast or rabbit muscle. We report here that the antibiotic has different effects on the three classes of glyceraldehyde-3-phosphate dehydrogenases that occur in spinach leaves, and we demonstrate its high specificity for the glycolytic enzyme.

We studied the enzymes of spinach because higher plants contain three different types of dehydrogenases which have glyceraldehyde-3-phosphate as substrate. Two of them are phosphorylating enzymes[7,8], distinguishable because one functions in glycolysis, is NAD-specific and located in the cytoplasm (EC 1.2.1.12), while the other is located in the chloroplasts and functions in the dark reactions of photosynthesis, being active with both NAD and NADP (EC 1.2.1.13).

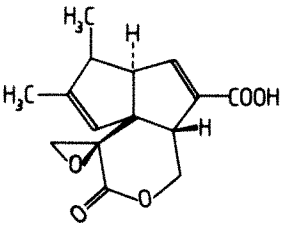


Fig. 1 Structure of pentalenolactone E (refs 4 and 5).

The third catalyses an irreversible and phosphate-independent reduction with NADP as cofactor and is located in the cytoplasm (EC 1.2.1.9; ref. 9).

Pentalenolactone was isolated from the fermentation broth of *Streptomyces arenae* strain Tü 469 by chromatography on XAD-2 resin, silica gel and Sephadex LH-20 as before[6]. Glyceraldehyde-3-phosphate dehydrogenases from spinach were enriched and partially purified, as described by Pupillo *et al.*[7], by ammonium sulphate fractionation, acetone precipitation, gel filtration, and separation of different enzymes by DEAE-cellulose–chromatography. (Rabbit muscle enzyme and all substrates and cofactors were purchased from Boehringer, Mannheim.)

As Fig. 2 shows, the inhibition of the two phosphorylating enzymes by pentalenolactone was time-dependent. Enzymes were incubated for various times with the antibiotic at room temperature before substrate was added. The activity of both enzymes declined rapidly during the first 2 min, but about 15 min of incubation were necessary for maximal inhibition. We therefore adopted this incubation time in subsequent experiments. Figure 3 shows the concentration dependency of the action of pentalenolactone. Compared with the rabbit muscle enzyme—the most sensitive tested so far—the cytoplasmic NAD-specific spinach enzyme required six times as much antibiotic for 90% inhibition. As Table 1 and Fig. 3 show, this is about the same amount needed to inhibit other glycolytic glyceraldehyde-3-phosphate dehydrogenases. Thus with respect to sensitivity to pentalenolactone, the glycolytic enzymes of the different species belong to the same class. On the other hand, the

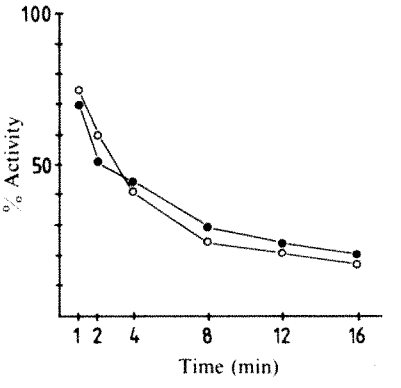


Fig. 2 Time dependency of glyceraldehyde-3-phosphate dehydrogenase inactivation by pentalenolactone. The enzymes were assayed in 0.1 M imidazole buffer, *p*H 7.4, containing 5 mM EDTA, 5 mM mercaptoethanol, 5 mM sodium arsenate, 2.5 mM coenzyme, and 0.15 IU enzyme in a total volume of 2.1 ml. Pentalenolactone E at a final concentration of 7 μM (chloroplast enzyme) or 1.5 μM (cytoplasmic enzyme) was added in 100 μl of methanol. In a control experiment with 100 μl pure methanol it was assured, that methanol does not disturb enzyme activity. The assay was started by addition of 250 μM (final concentration) D-glyceraldehyde-3-phosphate and the absorbancy change at 340 nm was measured for 1 min. The ordinate shows the incubation time before substrate addition in minutes. ○, Cytoplasmic enzyme; ●, chloroplast enzyme.

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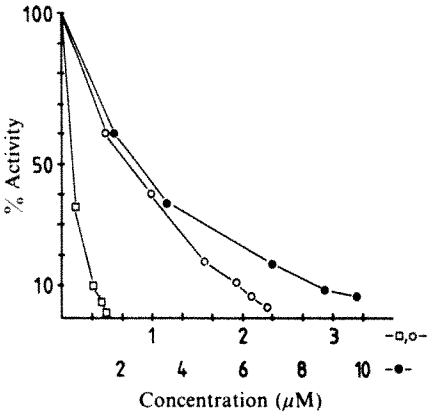


Fig. 3 Concentration dependency of pentalenolactone action. Assay conditions and enzyme concentrations were as detailed in Fig. 2, except for the non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (not shown, see text), when sodium arsenate was omitted. Incubation time before substrate addition was 15 min. □, Rabbit muscle enzyme; ○, spinach cytoplasmic enzyme; ●, spinach chloroplast enzyme. Note the change in scale on abscissa between glycolytic enzymes and chloroplast enzyme.

concentration of antibiotic needed to inhibit the chloroplast enzyme to the same degree is about 25 times those needed to inhibit the rabbit muscle enzyme. Thus, the chloroplast enzyme belongs in another group, as expected from its more variable cofactor dependency. To ensure that the NADP used in the chloroplast enzyme assay was not interfering with inhibition, some assays were done with NAD. The activity of a constant amount of chloroplast enzyme with NAD was about two-thirds of that obtained with NADP, but percentage inhibition with a controlled amount of pentalenolactone was the same. Thus, in a 2.5-μM assay solution the activity of the enzyme with NAD decreased 44% and the activity with NADP 50%. With a 3.5-μM solution 38% of activity was left in both cases. Furthermore, we confirmed that the protein-free plant extract contained nothing that interfered with the action of pentalenolactone. The third glyceraldehyde-3-phosphate converting dehydrogenase of higher plants, the nonphosphorylating enzyme, could not be inhibited to a significant degree by pentalenolactone concentrations up to 150 times that needed to inhibit completely the same amount of rabbit muscle enzyme.

Table 1 Relative sensitivities of different glyceraldehyde-3-phosphate dehydrogenases (GAPDH) to inhibition by pentalenolactone

Enzyme	Relative sensitivity
NAD-GAPDH, rabbit muscle	1*
NAD-GAPDH, *E. coli*	1.4*
NAD-GAPDH, yeast	3.2*
NAD-GAPDH, spinach	5.7
NAD(P)-GAPDH, spinach	24.5
NADP-GAPDH (nonphosphoryl. spinach)	>150

Sensitivities are expressed as concentration for 90% inhibition divided by concentration for 90% inhibition of muscle enzyme.
* Values from ref. 10.

Our results show that pentalenolactone is a universally applicable inhibitor of glycolysis. The difference in sensitivity to the antibiotic, shown by the three plant enzymes which function in different pathways, stresses the specificity of its action. The binding site of pentalenolactone to glyceraldehyde-3-phosphate dehydrogenase is not known, but Hartmann[10] has shown that the inhibitor strongly interferes with the binding of glyceraldehyde-3-phosphate to the rabbit muscle enzyme. Thus it seems possible that the substrate-binding part of the enzyme's active site is blocked by the inhibitor. The glycolytic enzymes from various organisms, having the same substrates and cofactors, and consequently a very similar active site, also show the same sensitivity to pentalenolactone. A minor change in the active site, indicated by the ability of the chloroplast enzyme to use NAD as well as NADP, is sufficient to decrease the enzymes' sensitivity to pentalenolactone considerably. Because of the epoxide structure of pentalenolactone E, its irreversible binding shown[10] for the rabbit muscle enzyme and also indicated for the spinach enzymes by the time-dependency of inhibition, and its interference with substrate binding, we conclude that it is covalently bound to the essential thiol group known to react reversibly with glyceraldehyde-3-phosphate during the catalytic cycle. The identification of the pentalenolactone binding site is in progress in our laboratory. We further speculate that the nonphosphorylating plant enzyme, which converts glyceraldehyde-3-phosphate to 3-phosphoglyceric acid, and is completely insensitive to inhibition by pentalenolactone, has no essential sulphydryl group in its active site.

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Erratum

In the article 'Tholins: organic chemistry of interstellar grains and gas' by C. Sagan and B. N. Khare, *Nature* **277**, 102–107, the following errors, generated at *Nature*, during editing, should be corrected: In paragraph 2 line 2, for H_2C read H_2S. In paragraph 3 line 11, for Sagan[8] read Sagan[10]. In paragraph 4 line 1, experimentally[9] should read experimentally[11]. In Table 2 the molecules listed, not the Table itself, are courtesy of Professor L. E. Snyder. The reference for the column labelled 'Gas phase spark' should be 37 not 30, and the reference for the column labelled 'Interstellar frost' should be 11 not 9. the last four lines of Table 2 should read:

Molecule

Propionitrile

$CH_3CH_2C{\equiv}N$

6. Cyanodiacetylene Pentenenitrile-H etc.
$CH{\equiv}C{-}C{\equiv}C{-}C{\equiv}N$
8. Cyanotriacetylene Benzonitrile ring breakage-H
$CH{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}N$
10. Cyanoctatetrayne
$CH{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}N$

In paragraph 6 the penultimate sentence should read: 'The fit of four spectral features between 3 and 4 μm to an accuracy of 0.3 μm will occur by chance with a probability 10^{-8}.' In paragraph 9 line 13, for tholins[33] read tholins[41]. In paragraph 13 line 3, for spluttering read sputtering, and in the same paragraph line 5 should read 'positive ion-molecule reactions may produce the simplest inter-'. In paragraph 15 line 11, for induces[34] read induces[38]. In paragraph 16 line 6, the name of H. C. van de Hulst is misspelled. In reference 5 the authors of the paper in *Astrophys. J. Suppl.* are C. Sagan and E. E. Salpeter.

Corrigendum

In the letter 'Combined effects of adrenaline and insulin on active electrogenic Na^+–K^+ transport in rat soleus muscle' by J. A. Flatman and T. Clausen, *Nature* **281**, 580, in Table 3 the units for serum insulin concentration should read $\mu U\ ml^{-1}$.

reviews

Good food for all

Kenneth Mellanby

Food for the Future. By K. Campbell. Pp.178. (University of Nebraska Press: Lincoln and London, 1979.) £8.75.

FOR some years those whom the popular press call ecologists have been telling us that mankind is doomed to perish from starvation. They say that we are not producing enough to feed the present world population of four thousand million, and that the shortage of energy and other resources, and the damage intensive farming is doing to the environment, means that in the future we will produce less, not more food. As the population is expected to be over six thousand million by the year 2000, and as it will probably increase further after that, the future seems desperate. Professor Keith Campbell believes that these doomsday men are "just plain wrong".

He is, of course, not the first to say this. Many agricultural scientists have suggested that, even without fundamental advances in farm practice, vastly greater productivity is possible in many parts of the world. The most striking report, published in 1975 by P. Buringh, H.D.J. van Heemst and G.J. Staring of the Agricultural University, Wageningen in the Netherlands, shows how the world's cereal production might be increased forty-fold. This would feed any possible population increase for many years to come.

Of course everyone, including Professor Campbell, must agree with the Malthusian view that if the world population continues to increase there must eventually be more than can be fed, but there are reasons for believing that this situation is a long way off, or, more hopefully, that population growth will be contained at a satisfatory level (as far as food production is concerned). This view is set out here, using familiar data, based on the fall in birth rate in both developed and developing countries. It is suggested that the most likely estimate is that the world population will stabilise at about eleven thousand million at the end of the twenty-first century; if so there is little doubt that they can all be fed.

Professor Campbell discusses the problems which will affect future agricultural productivity. He shows that these are of two types. First we have the scientific and agricultural problems — how much food can we produce; will there be land, water, energy and other resources; can the land sustain the present pressure? Secondly we have the economic and political problems — will farmers make a decent living; or will economic and social pressures make them work below their maximum level of productivity?

The first set of problems are dealt with briefly and in a familiar way. Even the most intensive systems use only a tiny fraction of the energy used by man in his other activities, so there should be enough for food production. Global supplies of fertilisers, including phosphate, are adequate for centuries. There is little evidence that food farming, no matter how intensive, is doing anything else than increasing the land's fertility.

Professor Campbell believes that productivity can be greatly increased if research is better organised to deal with the practical problems of the least productive countries. He is critical of much present-day research, particularly in universities, and complains that it is often divorced from the advisory ("extension") services. This view will be endorsed by many older agriculturalists in England and Wales, who regretted the post-War reorganisation which set up the National Agricultural Advisory Service (NAAS), taking advisory work from the universities, cutting off the research workers from the farmers, and producing the usual pullutation of unproductive bureaucrats. Nevertheless I fear that Campbell may be guilty of hubris, for assuming that more relevent research will certainly produce results which will improve food production. Unfortunately we cannot foretell the outcome of original research. But it will probably do some good, even if not as much as is here suggested.

The most interesting sections of this book are those which deal with the other constraints reducing potential food production. The environmentalists who wish to go back to peasant farming are given short shrift; Campbell believes that modern techniques, including chemicals, must be used efficiently, and that there need be no damage to the environment (even if some wildlife may be exterminated). More serious consideration is given to economic and political policies which encourage low productivity. Price control, quotas and other measures adopted by governments to influence agriculture receive severe and, to me, convincing criticism.

It is clear that poverty, not low food production, is the main cause of hunger in the world today. Wheat prices rose in 1973 because Russia had the money to buy a larger than usual share of the world surplus, thus pricing the poorer countries out of the market. This grain was bought to feed pigs, which had more economic power than starving peasants in the third world. Professor Campbell optimistically suggest that economic growth, world wide, will make increased food production profitable. Some readers will be more sceptical, but they will agree that it will be wise for greater food production, with greater self sufficiency, to be encouraged in poor countries, where internal food movements will be easier than between different sovereign states.

Incidentally, as a good economist, Professor Campbell wishes to give the customer what he wants. He has no time for the suggestion that the amount of animal protein in consumer diets should be reduced. Nevertheless it is realised that where intensively-kept livestock is fed on food which man could consume, more than half the protein and over 90% of the energy is wasted. Today world production is already sufficient to give more than the present population a nutritionally adequate, though dull, largely meatless diet. While we have surpluses, there is no reason why they should not be used to produce luxury food for the richer nations. Whether the rest of the world will ever be able to afford such diets is, in my opinion, doubtful. I am comforted by the knowledge that in a real crisis we could reorganise our feeding habits to ensure that the world's population could be adequately nourished. I only hope that Professor Campbell is justified when he says that "the world can look forward to substantially better diets in the future than were ever enjoyed in the past". □

Kenneth Mellanby was on the staff of Rothamsted Experimental Station from 1955-61, and is author of Can Britain Feed Itself?

Nuclear magnetic resonance

NMR and the Periodic Table. Edited by Robin K. Harris and Brian E. Mann. Pp. 459. (Academic: London and New York, 1978). £35.

THE great bulk of nuclear magnetic resonance work has been done on the nuclei of hydrogen, fluorine,phosphorus, carbon, boron and nitrogen, but most elements of the Periodic Table have at least one nuclide with a magnetic moment. Although quite a lot of work on many elements has been done in a few laboratories in the past twenty years, it is only relatively recently that commercial instruments with good enough resolving power and sensitivity have encouraged their wide use. In this book the editors and an authoritative and experienced group of authors have gathered together most of the work described so far in the literature, together with some unpublished work. The commonly studied nuclei mentioned above are dealt with only briefly and most of the book is devoted to work on the rest of the Periodic Table. The result is a remarkably comprehensive and fascinating account of an extraordinary range of chemical studies using chemical shifts, nuclear relaxation times, and scalar coupling constants.

The chapters on the various groups of elements also provide tabular data culled from the literature and presented in a convenient way which is likely to be valuable to those working or planning to work on those nuclei; and the coverage of the literature seems to be remarkably comprehensive.

There is a short account of experimental methods, and brief chapters to remind the reader about the theory of the chemical shift, nuclear spin coupling and nuclear relaxation. These are not intended for the beginner nor are they authoritative accounts, but are very useful for reference from time to time when reading other parts of the book.

Dr Harris and Dr Mann are to be congratulated on producing such a useful and interesting book and on having persuaded so many co-authors to cooperate in writing such a compendium.

Rex Richards

Sir Rex Richards, formerly Dr Lee's Professor of Chemistry at the University of Oxford, is now Warden of Merton College and Vice-Chancellor of the University of Oxford, UK.

Geochemical mapping

Regional Geochemical Atlas: Orkney. Compiled by the Institute of Geological Sciences. (Institute of Geological Sciences Bookshop: London, 1979.) £35. *An Introduction to the National Geochemical Data Bank.* (IGS Computer Application Report No. IGS/CAR/78/2.) Second edition. (Institute of Geological Sciences Bookshop: London, 1979.)

THE acquisition of regional geochemical data, based on the collection and analysis of steam sediment samples, had its origin in mineral exploration geochemistry. Individual mining and prospecting organisations would undertake regional geochemical surveys in order to delineate those parts of an exploration area which had the highest probability of containing mineral deposists.

The areas covered by such surveys varied from a few square kilometres to tens or even hundreds of thousands of square kilometres, and great success was achieved with the technique. The data obtained, however, seldom became widely available due to the competitive nature of the mining industry, and as a result some areas in well mineralised environments were surveyed many times. Certain areas in the Irish Republic, for example, are believed to have been surveyed five or six times.

Subsequently, further applications of regional geochemical data have been discovered. The relationship between certain human and animal ailments and the occurrence of abnormally high, or abnormally low levels of certain specific elements in the soils and vegetation of certain areas has been appreciated. The influence of the levels of many elements upon the success or failure of some crops has also been investigated. There can be little doubt that many new uses of regional geochemical data will be discovered in the future, especially if such data become freely available. The publication of the *Regional Geochemical Atlas* by the Institute of Geological Sciences will be especially welcome in this connection.

In the first instance, the atlas will cover the whole of Scotland. The atlas is not the first such publication to appear. As long ago as 1973, the Applied Geochemistry Research Group at Imperial College, London, produced its *Provisional Geochemical Atlas of Northern Ireland;* the somewhat less ambitious *Reconnaissance Base Geochemistry of Parts of the Counties Wicklow, Wexford, Kildare and Dublin* was published by The Geological Survey of Ireland in 1977; and the Applied Geochemistry Research Group published the impressive *Wolfson Geochemical Atlas of England and Wales* (Oxford University Press: Oxford) in 1978.

In the IGS atlas, the distribution of 15 elements in stream sediments is illustrated in the same number of maps, plus another showing the distribution of uranium in stream waters. Other maps illustrate the relief, geology and mineralisation of the area, together with data collected from a smaller number of sites on the *p*H and conductivity of the streams themselves.

The maps, on a scale of 1:250000, were produced by automated cartographic methods which were developed jointly by the IGS and the NRTC Experimental Cartography Unit. The level of the relevant element at each sample site is illustrated graphically by the use of lines which are proportional in length to the concentration of the element in the sediment, the lines for each element being orientated in a specific direction.

This method of presentation has the advantage that the results for each sample site are clearly represented, as distinct from the smoothed 'grey-scale' approach adopted by the Imperial College group. Whether or not this manner of data presentation can be considered to be entirely successful, however, is a matter of personal opinion. The reviewer did not find it completely satisfactory, especially where several sample sites occur in close proximity, so that individual lines tend to merge into a single mass which is difficult to interpret.

The prospect of having such regional geochemical maps freely available in the future is clearly one which will increase the scope of geochemical studies. Indeed, one can imagine the time in the future when geochemical maps are regarded as being as basic a requirement as geological maps are today. Nevertheless, many areas will have been the subject of geochemical studies other than those presented in such atlases,and the need to make the data from these studies both available and accessible is also a matter that calls for attention.

The Regional Geochemical Atlas series is part of the Department of Industry's wider Regional Geochemical Reconnaissance Programme under contract to the Natural Environment Research Council. This programme includes the IGS National Geochemical Data Bank which holds data compiled in the production of the geochemical atlases, as well as regional geochemical data from other sources. The establishment of this Data Bank is also to be welcomed, as the use of a central computer for the storage and management of geochemical data could obviously lead to a more effective use of information available.

Data to be accepted initially will be related to igneous, sedimentary and metamorphic rocks, drainage samples (that is, stream sediment samples) and soils, the results for each type of material being stored separately on the basis of geographical location. The bank will be under the control of a manager, who will be responsible for maintaining a suitable level of quality of the data in the bank. Before being archived, data will also be examined by an Advisory Committee consisting of

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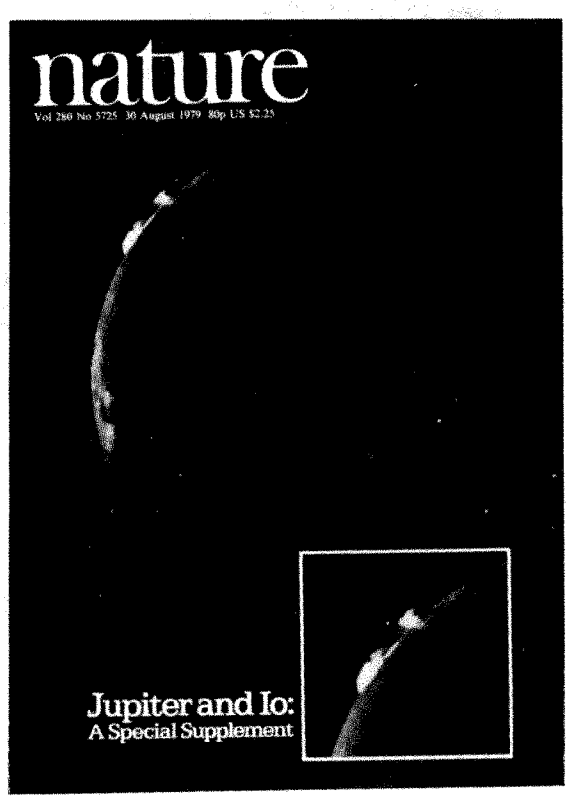
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specialists in each particular field; and during the archiving process, a variety of checks will be applied in order to maintain quality control.

It is perhaps the question of quality control which presents the most important point of contention over the question of a data bank of this type, for not only will the quality of data received vary from project to project, but the level of quality of data that is acceptable to the users of the bank will also vary. Thus a data set that is rejected by the bank as being of unsatisfactory quality, would be denied to a user to whom it might be quite acceptable. It would perhaps be more satisfactory if data sets were accepted into the bank on the basis of an accompanying quantitative statement of data quality, which would enable the user to select or reject the results of any particular project.

These two publications, therefore, may be seen as heralding a new approach to the study of geochemical problems. It is good to see the IGS leading the way in these important matters.

C. H. James

C. H. James is Senior Lecturer in Economic Geology at the University of Leicester, UK.

Life through Earth's history

The Encyclopaedia of Prehistoric Life. Edited by Rodney Steel and A.P. Harvey. Pp. 218. (Mitchell-Beazley: London, 1979.) £12.

THE editors of an encyclopaedia have a very exacting task in ensuring that their volume lives up to the name, not only in its facts but in its balance and overall content. To ensure factual correctness a formidable array of palaeontologists is here listed as contributors, totalling some 23, most of whom have an international reputation; some of them would no doubt figure on a list of the world's leading palaeontologists, but they hardly constitute such a list, as the dust-cover would have us believe.

The editors are Rodney Steel, a journalist, and Anthony P. Harvey, a librarian; both clearly have an absorbing interest in palaeontology. In their role as editors, however, they have failed to make full use of the talents of their team of contributors, several of whom only have one subject entry, and rely very heavily on their own contributions, which amount to almost 40% of the entries in the book. Rodney Steel contributes on vertebrate palaeontology (in considerable detail), on localities and formations (almost exclusively dealing with vertebrate fossils and omitting, for instance, Ediacara, the Bundenbach Slate, and so on), and on various other subjects with considerably less success. Anthony Harvey uses his bibliographical knowledge to provide biographical information on pioneering palaeontologists, and short sections on institutions with palaeontological museums. Both these lists have some notable omissions but one wonders whether they have place at all in a book on prehistoric life.

There are three main non-editorial contributors, J.W. Franks on plants and L.B. Halstead and R.J.G. Savage on vertebrates. These three provide authoritative reference material which is amplified by beautifully executed line and stipple drawings which characterise the whole book.

The composition of the rest of the contributing team and the use to which they have been put, raise many queries. One questions the logic of using five vertebrate palaeontologists (in addition to Rodney Steel who writes extensively on vertebrates). There are five workers on fossil arthropods, only one of whom has contributed on this phylum (which merits a meagre nine entries); of these five, three work on trilobites but none has contributed to the "trilobite" entry. There are two authors who have worked on the Burgess Shale fauna, but neither has written under that heading. Some 17 of the contributors have produced in total well under 20% of the entries.

The standard of the entries by the professional palaeontologists is high, but it is very clear that some (at least) of the authors have not had the opportunity to check proofs of their own sections. Under "echinoderms", for instance, blastoid hydrospires appear as "hydropores" and "hydrospores", and an inexcusable editorial insertion of "(spore cases)" appears after the word "theca".

The book is excessively vertebrate-oriented; to widen its appeal, some leaning towards the more conspicuous faunas is excusable. Here though, reasonable limits are exceeded. Over a quarter of the diagrams are of mammals (some groups are dealt with in great detail, so that elephants, mammoths and mastodons have no fewer than eleven large size drawings); another quarter is of reptiles, and a further fifth of other vertebrates. With limited space left for plants and invertebrate groups, the choice of diagrams is sometimes curious. For instance, many echinoderm classes are unfigured, yet there are two large drawings of Lower Jurassic ophiuroids and four views of one species of echinoid.

Molluscs too are poorly served. There is no entry under the heading "cephalopods", although ammonites and belemnites do appear under separate entries. Nautiloids and early ammonoids are described under "ammonites", but coleoid groups such as octopuses, cuttlefish and squids (the latter two at least with a healthy fossil record) do not merit entries. There are no diagrams of such molluscan groups as scaphopods, polyplacophorans or monoplacophorans, and no mention of segmentation under the latter or under *Neopilina* (mentioned separately, without cross-reference, under "Living fossils").

One of the problems the user of this book must face is inconsistency. Here a firm editorial hand should have spotted these probems. Thus Stromatoporoids and Stromatoporata are treated (by the index at least) as separate organisms; this confusion is compounded by a figured bryozoan being labelled as *"Stromatopora"* rather than *Stomatopora*. Bivalves are referred to as such in the text in some places, and as pelecypods in others — only the latter rates an entry in the index. Ranges of organisms vary too — the first belemnites are stated (correctly) to occur in the Carboniferous under "Belemnites", but in the Permian under the entry for that system. Ranges of some fossil groups are incorrectly stated.

Each geological system has an entry, and most are accompanied by a small time-scale chart showing subdivisions of that system. These are clearly not the authors' work, but are culled from a well-circulated colourful chart which is, unfortunately, full of errors. These are perpetuated in the book, but some new errors have crept in too.

Blackie

Biochemical Systematics and Evolution

Andrew Ferguson, Queen's University, Belfast

This is a review of the most recent developments in biological systematics, written at a level suitable for undergraduates and postgraduates in the biological sciences. The author lays special emphasis on the technique of gel electrophoresis. Throughout, he selects examples which best illustrate particular concepts or applications, although most examples concern animals and particularly the vertebrates.

Suggestions for further reading are provided for each chapter and there are extensive bibliographic citations in the text.

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In addition to the numerous line drawings there are fourteen glossy colour charts. Eleven feature vertebrate phylogenies in considerable detail, with many genera mentioned. In contrast two charts of plant and invertebrate phylogenies are very poor and largely at phylum level; the latter lacks a time-scale.

The over-emphasis on vertebrates throughout the book reaches ludicrous proportions when, under the heading "Zone fossils", vertebrate groups receive more space than invertebrates.

There is a glossary which is again heavily vertebrate-oriented. The geological entries are sometimes appallingly incorrect and could not have been written by a professional geologist; the authorship of this section is not given.

The book is presumably aimed at the layman who wants to learn something about life through Earth's history. If he seeks information on extinct vertebrates, then this may be the book for him, but it will otherwise have a limited appeal. In spite of the excellence of the great majority of the drawings and its wealth of detailed information on vertebrate fossils, its inaccuracies and inconsistencies together with, for most readers, an unacceptable imbalance in its content, render the title *Encyclopaedia* a pretentious one. Though it is attractively laid out and handsomely bound, at £12 for 218 pages it is an expensive way to cover your coffee-table.

J.C.W. Cope

J.C.W. Cope is Senior Lecturer in Geology at University College, Swansea, UK.

Artificial intelligence at MIT

Artificial Intelligence. (Two volumes). Edited by P.H. Winston and R.H. Brown. Pp.492 and 486. (MIT Press: Cambridge, Massachusetts, and London, UK, 1979.) £16.25; $26 each volume.

AMONG the new scientific arts which have arisen since World War II, artificial intelligence must surely be among the most consequential and the most widely misconstrued. Yet its practitioners are neither diffident nor ambiguous in defining their field of study. "Its central goal", say P.H. Winston and M. Brady in their foreword to a new series from MIT Press, "is to make computers intelligent, both to make them more useful and to understand the principles that make intelligence possible".

The first fruit of the MIT Press series is a two-volume collection of 29 papers by graduate students, young post-doctoral workers and senior staff at the Massachusetts Institute of Technology. They are scattered over a variety of AI-related topics and first appeared, usually in longer form, as technical memos. The editors, P.H. Winston (who now directs MIT's AI Laboratory) and R.H. Brown, have grouped them into sections as follows: in Volume 1 — expert problem solving, natural language understanding and intelligent computer coaches, representation and learning; in Volume 2 — understanding vision, manipulation and productivity technology, computer design and symbol manipulation.

The reader who hopes for a true perspective view, a shining framework of deductively related principles and laws, will recoil from this congeries. Thus some pre-Newtonian seeker after a science of mechanics might have recoiled from Leonardo's notes on motion, weights, impact and moments of force. Technology's most thrilling advances commonly take place through spasms of untidy thought and activity. While waiting for the orderly abstraction of the theoretician, we may have to quarry general principles from the disorder of notes made in the heat of the struggle by the protagonists themselves. The editors of this book do, however, give conscientious aid to the quarrying process. Very full summarising prefaces precede each section, with an additional shorter note on each chapter. Newcomers to the field will be particularly grateful where specific AI techniques are listed and sign-posted to individual papers.

Some contributions, such as Stallman and Sussman on mechanised circuit analysis, represent deep and thorough finished studies. Others record brave first skirmishes with problems which will not fall before many such assaults have spent themselves. Johan de Kleer's study of qualitative and quantitative reasoning about roller-coaster mechanics is a stimulating example, and Winston's exercise in machine learning through simile raises hope of good eventual solutions in an extremely difficult area. In Marvin Minsky's "The Society Theory of Thinking", in which "the mind is viewed as an organised society of intercommunicating 'agents'", bravery shades into audacity, and the lines of contact with neurobiological fact are stretched exceedingly thin. Yet speculation may still in this field engender aids for the practitioner, as Minsky's earlier "frames" proposal has already amply shown.

Of all the difficult problems addressed by artificial intelligence workers, common consent gives the computational analysis of vision pride of place. Among the six vision papers of the second volume, David Marr's is outstanding. From the strenuous detail and breadth of this attack, methodological lessons can be learned. One of these is summarised by Winston and Brown: ". . . it is wrong to begin with devotion to some particular type of algorithm with a view toward finding some problem that it will solve". These words reflect AI's recent change of emphasis for which the MIT school can claim chief credit, namely that intelligent behaviour can only be conjured from a sufficiently rich and massive base of empirical knowledge about the specific domain appropriately organised in machine memory.

Artificial intelligence has thus followed an opposite course to that of the mechanical crafts, which in their early days lost themselves in the properties of specific domains. It was left to Leonardo to make the all-important step of abstracting the concept of a generalised mechanism — for example, for converting between rotary and reciprocating motion — which could then be made to function in any of a variety of different specialised machines. With AI it went the other way, and for 15 years most of us were hypnotised by the promise of general mechanisms — for heuristic search, for deductive reasoning, for machine learning, and the like — as though these universals could accomplish every problem-solving task without the additional need for building in carefully articulated representations of knowledge.

Indeed for a time the MIT school went to the opposite extreme and maintained the exuberant position that domain-specific knowledge should be inextricably interwoven ("procedural embedding") into the substance of the algorithmic mechanisms themselves, instead of maintaining, as we do today, separate "knowledge bases" to which these more general mechanisms can have access. It has now been sufficiently demonstrated that the resulting loss of modularity is fatal to incremental construction and flexible adjustment of an intelligent program's packet of skills. Incremental properties are quintessential. It is refreshing to note from these volumes that the point is now taken, including at MIT.

Space does not allow mention of many other rewarding views to be had of AI people at work. Lozano-Perez's discussion of the problem of telling a robot how to move its hand illuminates the intrinsic complexity of a task at which humans are hereditary Grandmasters. The software technologist will find Hewitt on control as message-passing a source of provoking novelty. Hewitt's chapter closes a book which will irritate purists and antagonise some professionals whose orientation is to Meccas other than MIT. But to those who wish to take a dip into the fast-flowing stream of an active and authoritative laboratory, the publication of this book offers a unique opportunity.

Donald Michie

Donald Michie is Professor of Machine Intelligence at the University of Edinburgh, UK.

obituary

Lew Kowarski

LEW KOWARSKI was the last survivor of the French team that, immediately after the discovery of uranium fission, explored the chances of a nuclear chain reaction. The paper by Otto Hahn and Fritz Strassmann reached Paris about 16 January 1939 and Frédéric Joliot at once saw its tremendous implications. Hans von Halban offered his experience of neutron physics and suggested that Lew Kowarski should join the team. Within weeks they had proof that neutrons, in causing uranium nuclei to split, produced further neutrons; by April they were sure that under suitable conditions those further neutrons would produce further fissions and vice versa, a chain reaction that would lead either to an explosion or to the generation of useful power if the reaction was controlled.

Born in 1907 in St. Petersburg (now Leningrad), Lew had an insecure childhood in a divided household, as son of a Jewish businessman and a Ukrainian singer. At the age of 12 he was smuggled from the Russian revolution to Vilna (in what later became Poland) across the remnants of the German army, some months after his father had gone there. He grew into a giant; musical though he was he had to give up his piano lessons when his fingers grew too thick for the piano keys; so it had to be science. With modest support from his father he studied chemical engineering in Belgium and France.

In 1929 he enrolled in the University of Paris, studying physics and chemistry from books and supporting himself by a job in a firm that made gas pipes, a job he kept part time until 1938. In 1931 he began work on a thesis on crystal growth in Jean Perrin's laboratory, made friends with his son Francis Perrin, and got in touch with Madame Curie, her daughter Irène and Irène's husband Frédéric Joliot just when the couple discovered "artificial" radioactivity at the end of 1933. That brought them the 1935 Nobel prize for chemistry, and Joliot then became professor at the College de France. Kowarski had already been his part-time unpaid assistant for a time; he now became a paid part-time secretary, "Joliot's little typist" as the huge Russian got nicknamed; and then he got a grant that allowed him to drop his other job, of designing gas pipe systems, about which he had just written a book. He still felt an intruder in spite of his doctorate, having got into science by the back door, at the late age of 31; but he was in.

The team agreed that, in publishing, no ideas should be attributed to individuals; but later it became clear that some of the best ones had been due to Kowarski, who combined inventiveness with meticulous logic. The neutrons were slowed down in water to make them better at causing fission, but many were absorbed before they did. Kowarski urged that heavy water be used, known to absorb hardly any neutrons. It has to be extracted from ordinary water at great cost, and one firm in Norway was doing that; in March 1940 their entire stock, about 40 gallons, was moved to Paris just before the Germans attacked Norway. During that transfer those aliens, Halban and Kowarski, were interned on two separate islands! But time was running out; in May France was invaded, and in June — as instructed by Joliot — Halban and Kowarski escaped to England with their families and the heavy water.

At the Cavendish Laboratory in Cambridge new equipment was built with help from local physicists, mainly under the guidance of Kowarski while Halban negotiated with the USA. The results were promising, but much more heavy water was needed, no longer obtainable from Norway. And why should the USA help Halban, with his German manner, who waved French patents and allied himself with ICI? He seemed more intent to compete after the war than to help win it.

With influential friends and fluent English (though Kowarski soon caught up) Halban assumed a dominant position. In 1942 he was sent to Canada to set up a laboratory; Kowarski, offered an inferior post, decided to stay in Cambridge, as did some of his co-workers. Late in 1943, with some US help promised, (Sir) John Cockcroft took over from Halban, and at last Kowarski got his way: he came to Canada with his team to build a heavy-water reactor. He did it in less than a year; it was the first nuclear reactor outside the USA where Enrico Fermi had achieved the first self-sustained chain reaction in 1942.

After the war Kowarski would have liked to go back to England, where in Cambridge he had felt at home from the first day; but he felt in duty bound to return to France. Under Joliot, scientific head of the Commissariat a l'Energie Atomique, he began to prepare for nuclear power in earnest. Again he built a small heavy-water reactor, both for experimentation and prestige, which went critical late in 1948; nobody knew that the USSR had a reactor going two years before that. Bigger reactors were on the drawing board; would their main product be power or plutonium? Joliot, now an open communist, publicly refused to make atomic weapons and in 1950 was dismissed from the Commissariat. Kowarski, still working for it part-time, found a new career with CERN, nursing that young European Centre for Nuclear Research. In 1948, his first marriage broken up, he had married Kate Freundlich, daughter of a German scientist; in 1954 they settled in Geneva for good.

As CERN grew his work was gradually taken over by others; in the end he concentrated on data processing with computers, whose importance he saw earlier than most; the device for measuring bubble-chamber tracks that Paul Hough and Brian Powell built with his guidance was used all over the world. A few years' working and travelling for ENEA (the European Nuclear Energy Agency) restored his freedom to visit the USA, denied for years because of his past work with that notorious communist Joliot, and enabled him to hold visiting professorships, two extended ones at Purdue University and shorter ones at Austin (Texas) and at Boston University. In his last years he worked to warn the public against too headlong a growth of nuclear power.

Outwardly like a big jolly Russian peasant, he had a nimble mind and a fine command of both French and English. Honest to the point of bluntness, he made some enemies and many devoted friends. His interest gradually shifted from science to the philosophy and strategy of scientific organisations. Overwork damaged his health, and the single kidney he was born with began to fail years ago; on 27 July he died in a Geneva hospital, cared for by his wife and his daughter Irene from his first marriage. That he survived so long was due to medical art, his own tremendous will, and the care of his calm and gentle Kate.

Otto R. Frisch

Percy Brian

PERCY WRAGG BRIAN died at the age of 68 on 17 August 1979. He was one of the leading botanists of the day with a wide range of interests.

Percy Brian graduated from Cambridge in 1931 with first class honours in the Natural Science Tripos, was awarded the

Frank Smart Studentship as the best student of his year and remained at Cambridge to take a PhD, awarded in 1936. He was awarded an ScD in 1951. His professional career started at Long Ashton Research Station in 1934 and he joined ICI in 1936, where he remained until 1963 — first at Jealott's Hill and later at the Akers Laboratories. He returned to academic life in 1963 as Regius Professor of Botany in Glasgow; in 1968 he moved to Cambridge as Professor of Botany, where he stayed until his retirement in 1977. He was awarded an honorary DSc by Hull University in 1978.

Brian's most productive years as a research worker were spent at the Akers Laboratories. Here he collected an active research group and enjoyed close collaboration with organic chemists who shared his interest in natural products. His research planning and management were faultless; he maintained his interest in a problem only as long as the results justified the work involved; he noticed potential developments peripheral to his central theme, but he did not pursue them until he could give them adequate backing; he did not fritter his energy on red herrings. Once he had launched a new line of research, he gave the workers themselves complete freedom to develop it. The result was a happy, well-integrated and purposeful group.

Brian's central interest as the Akers Laboratories was the influence of antibiotics on the balance of fungi in the soil; this involved collecting antibiotics from soil fungi, examining their effects on other fungi and on higher plants and trying to establish whether the same chemicals were produced in soils. Brian was not deterred by the magnitude and complexity of this problem. During the course of this work he noticed that although most of the antibiotics were generally toxic, a few, notably griseofulvin, were more specifically active against fungi and might have a potential in plant chemotherapy. Brian launched a programme to study this possibility some 10 years before the subject became respectable. Griseofulvin was never sucessful as a plant chemotherapeutant, but found a unique place in the treatment of mycoses in man and other animals, a possibility which Brian foresaw some years before the chemical was developed.

The chemical interactions of soil fungi included the activity of parasites as well as saprophytes. Among other parasites he studied the products of *Gibberella fujikuori* and isolated the active chemical, gibberellic acid, in large enough quantities to launch a new area of botanical research. The interactions between fungal parasites and their hosts, particularly as related to the balance of hormones in the plant, remained Brian's central research interest. He pursued these studies at Glasgow and Cambridge with the support of the Agricultural Research Council. The ARC Unit of Developmental Botany was established at Cambridge in 1969 with Brian as honorary director. Here hormone physiology was studied in both healthy and diseased plants.

Brian had less time for research in his university posts but he was known as a tower of strength in his departments and he and his wife, Meg, were untiring in their hospitality to students and to their colleagues.

In addition to research and teaching, Brian was unstinting in the time he would give to the substructure of his science. He was a member of the Agricultural Research Council, twice president of the British Mycological Society, president of the Association of Applied Biologists, the Society of General Microbiology and of the Cambridge Philosophical Society at the time of his death. He was vice-president of the Institute of Biology and served on the council of the Society for Experimental Biology. He also maintained his interest in plant pathology and was one of the chief organisers of the 1st International Congress of Plant Pathology. He was elected a Fellow of the Royal Society in 1958 and served on its Council from 1968 to 1970. He delivered the Leeuwenhoek Lecture in 1966. He was created CBE in 1975.

Brian was never a loquacious man and never pushed himself forward. However, he never refused help and advice when it was requested or needed and what he did say was to the point and well worth listening to. It was probably a measure of his greatness that so many people 'beat a path to his door.' He will be sorely missed by his friends and colleagues and particularly by his family, who must command our deepest sympathy.

S.H. Crowdy

Martin Prestige

MARTIN CALDER PRESTIGE, developmental biologist and Senior Lecturer in Physiology at Edinburgh University, died suddenly on 27 August 1979.

His death, at the early age of 44, removes from the world of developmental neurobiology one of its most incisive minds and able investigators. Educated at Cheltenham College and Trinity College, Cambridge, his basic scientific training was in physiology and led to his being awarded a Michael Foster Studentship. In 1958 he started research, with Patrick Merton, on the occurrence of initial collaterals on kitten motorneurons.

In 1963 Martin joined Arthur Hughes in work on the development of the amphibian spinal cord. This collaboration started in the Anatomy School in Cambridge, continued from 1964 at the Zoology Department in Bristol, and had a great and continuing effect on the general direction of his work. He took his PhD at Bristol in 1967; this was on the development of the nervous system to he hind leg of *Xenopus* tadpoles and it outlined what was to be one of his main research interests. He continued working in Bristol until he moved to Edinburgh as a Lecturer in Physiology in 1968. He loved Edinburgh and remained there, being appointed Senior Lecturer in 1975.

Two main themes run through Martin's work. The first concerns how the nervous system is put together in terms of cell types and cell numbers; and, with a slight modification of emphasis, this changes into the second theme, which concerns how the system is established in terms of adult functional connectivity.

In Martin's early work his friendship with Arthur Hughes shows most clearly, for he started from Hughes' observations on development in *Xenopus* and extended these brilliantly. In a series of papers starting in 1965 he analysed the phenomena of cell death during the development of the spinal cord and its sensory and motor connections. This was beautiful work, highly relevant to ideas that were then beginning to emerge on the role of central-peripheral relationships in the developing nervous system, and it continued up to his death, with a paper currently submitted for publication.

In 1975 the second main theme of Martin's work was illustrated by the publication, with David Willshaw, of their now well-known paper on theoretical aspects of the formation of retinotectal connections. This paper was very important in that it introduced, for the first time, a welcome touch of rigour into the study of how the eye connects with the brain. Up till then there had been, unbelievably, virtually no serious assesssment of the assumptions underlying the various mechanisms that had been proposed to account for the formation of retinotectal connections. Since then there have been several (and more comprehensive) papers on this subject, by others, but the initial credit must go to Prestige and Willshaw.

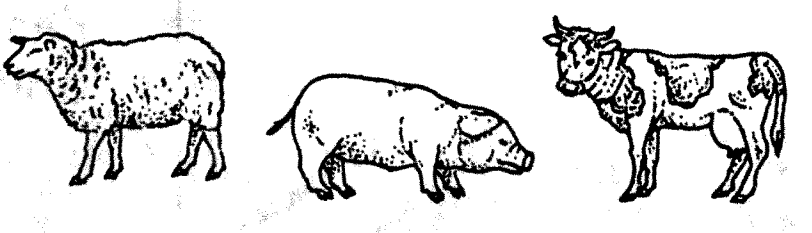
Martin was also a poet of considerable ability. He had, in his youth, been a mountaineer, and he maintained this interest until his death, in the form of an extensive collection of books on the Himalayas. His first wife, Anne, was killed in a tragic accident. He was married for a second time, to Lucina, in 1972, and their further years in Edinburgh were happy.

In the last year of his life Martin was much troubled by respiratory disability resulting from an illness in youth. Those who knew him over the years will miss him as a friend, as a distinguished scientist, as an inspiration to his colleagues and as an outstanding example of how to behave in the face of adversity.

R.M. Gaze.

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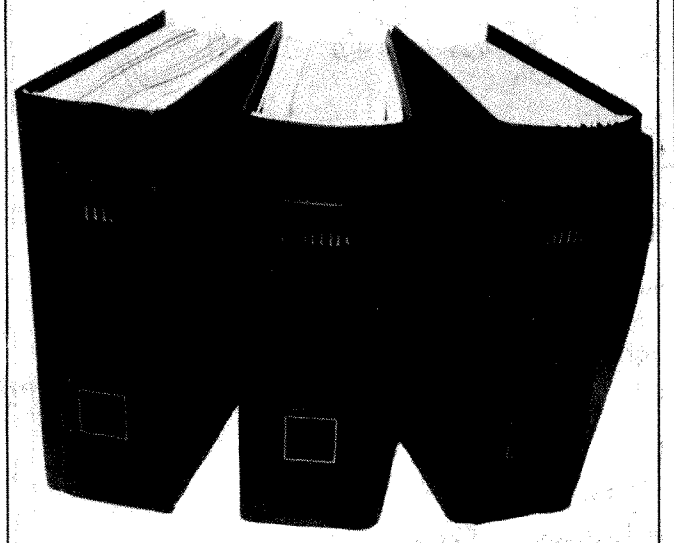
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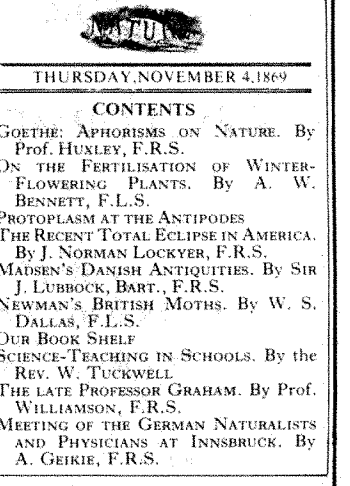
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21-25 January, **Laser Application,** Essex (The Liason Officer, University of Essex, Wivenhoe Park, Colchester, UK).

21-25 January, **Practical Spectral Analysis,** Sheffield (Dr H.K. Kohler, Industrial Liaison Officer, University of Sheffield, Dept of Mechanical Engineering, Mappin St, Sheffield, UK).

22 January, **Hazard Control and Planning in the Working Environment,** London (Prof. J.H. Martin, Dept of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

24 January, **Recent Advances in Bio-organic Chemistry,** London (Stanley Langer, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

30-31 January, **Neuroactive Peptides,** London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

31 January-4 February, **Yesterday, Today and Tomorrow of Indian Leather & Allied Industries,** Madras (Central Leather Research Institute, Adyar, Madras-600 02, India).

6-8 February, **3rd International Congress on Industrial Waste Water and Wastes,** Stockholm (Bo Goransson, Box 21060, S-100 31 Stockholm, Sweden).

7 February, **New Developments in NMR Spectroscopy,** London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

12-15 February, **Geological and Geophyical Consequences of Ridge Subduction,** La Jolla (American Geophysical Union, 2000 Florida Ave, N.W., Washington, D.C. 20009).

14 February, **Photosynthetic O_2 — evolving Prokaryotes: a current review,** London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

6 March, **Synthesis in Natural Product Chemistry,** London (Stanley Langer, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

10-14 March, **1980 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy,** Atlantic City (The Pittsubrgh Conference on Analytical Chemistry & Applied Spectroscopy, 437 Donald Rd, Pittsburgh Philadelphia 15235).

17-21 March, **Epidemiological Approach to Occupational Cancer,** Lyon (IARC, 150 cours Albert Thomas, 69372 Lyon Cedex 2, France).

18-20 March, **Genetic Control of Natural Resistance to Infection and Malignancy,** Montreal (Emil Skamene, Division of Clinical Immunology and Allergy, 1650 Cedar Ave, Montreal, Quebec, Canada).

23-26 March, **EURIM 4,** Brussels (Aslib, 3 Belgrave Square, London SW1, UK).

24-28 March, **Renewable Energy Technology for Development,** Strathclyde (Dr Malcolm Slesser, University of Strathcylde, Glasgow, UK).

25-27 March, **Electron Conduction in Biology,** Lancaster (Prof. C.F. Phelps, Dept Biological Sciences, Unviversity of Lancaster, Lancaster, UK).

25-27 March, **Dielectric Relaxation Spectroscopy,** Modane (Dr C.W. Smith, Dept of Electrical Engineering, University of Salford, Salford, UK).

24-27 March, **New Developments with Human and Veterinary Vaccines,** Israel (Ms A. Snir, Secretary OHOLO Conference, Israel Institute for Biological Research, PO Box 19, Ness-Ziona 70400, Israel).

24-28 March, **EUROCON '80,** Stuttgart (Prof. Dr W. Proebster, IBM Deutschland GMBH, Postfach 800880, D-7000 Stuttgart 80, FRG).

31 March, **Self-Medication: The New Era,** Arlington (Ms Jacqueline Maio, The Propreitary Association, 1700 Pensylvania Ave., N.W., Suite 700, Washington, DC. 20006).

31 March-1 April, **Drug Receptors and their Effectors,** London (Dr N.J.M. Birdsall, National Institute for Medical Research, Mill Hill, London NW7, UK).

Reports & Publications

UK & Ireland — October

Proceedings of the 1974 Ore Microscopy Summer School at Athlone. Edited by M.J. Oppenheim. (Special Paper No.3.) Pp.vi + 119. (Dublin: The Geological Survey of Ireland, 1979) (110

Council for National Academic Awards. Annual Report, 1978. Pp.84. (London: Council for National Academic Awards, 344-354 Gray's Inn Road, WC1, 1979.)

Health and Safety Executive. The Accident at Bentley Colliery, South Yorkshire, 21 November 1978, Report Pp.18. (London: HMSO, 1979) £1.25 plus postage.

Water Research Centre. Annual Report and Accounts, 1978/1979. Pp.32. (Henley-on-Thames: Water Research Centre, 45 Station Road, 1979.) (210

International Trade in Wildlife. By Tim Inskipp and Sue Wells. Pp.104. (London: Earthscan, 10 Percy Street, W1, 1979.) £2; $4.50. (310

Health and Safety Executive. First Aid at Work: Draft Regulations, Approved Code of Practice and Draft Guidance Notes. (Consultative Document.) Pp.21. (London: HSE Enquiry Point, Baynards House, 1 Chepstow Place, W2, 1979.) 50p. (410

Office of Population Censuses and Surveys. Abortion Statistics: Legal Abortions Carried Out under the 1967 Abortion Act in England and Wales. (Series AB No. 4) Pp.x + 64. (London: HMSO, 1979) £2.75 net. (410

Radioactive Waste. By L.E.J. Roberts. Pp.32. Glossary of Atomic Terms. Ninth edition. Pp.72. Nuclear Power: How and Why. Pp.9. (London: United Kingdom Atomic Energy Authority, 1979.) (510

The Common Agricultural Policy—Some New Thinking from Dr. Sicco Mansholt. (A translation from the Original Interview Conducted in the German Language.) Pp.18. (Haughley, Stowmarket, Suffolk: Soil Association, Walnut Tree Manor, 1979.) 30p. (810

The Development of Atomic Energy: Chronology of Events 1939-1978. Pp.50. (Harwell: UKAEA, Authority Historian's Office, 1979.) Available from UKAEA, 11 Charles II Street, London, SW1.) (810

Commission on Energy and the Environment. Review of Activities, 1978-79. Pp.17. (London: HMSO, 1979.) £1.25 (910

Measuring Colour. Second edition. Pp.30. (London: Tioxide International, Ltd., 10 Stratton Street, W1, 1979.) (910

Dementia in Old Age. Pp.72. (London: Office of Health Economics, 162 Regent Street, W1, 1979.) 35p. (1110

Department of the Environment: Central Directorate on Environmental Pollution. Monitoring the Marine Environment: The Way Ahead. (The Second Report of the Marine Pollution Monitoring Management Group, 1977-1978 . Pp23. (London: Department of the Environment, 2 Marshal Street, SW1, 1979.) 70p + 18p postage. (1210

Community Markets, No. 1, October, 1979. Edited by Margie Lindsay. Pp.1-16. (A Monthly briefing for businessmen on European Community developments.) (London: *The Financial Times,* 10 Cannon Street, EC4, 1979.) (1510

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 287, No. 1019: Morphology of the Wings, Legs and Tail of Three Coniferous Forest Tits, The Goldcrest, and the Treecreeper in Relation to Locomotor Pattern and Feedings Station Selection. By Ulla M. Norberg. Pp.131-165. UK £2.55; Overseas £2.76. Vol.287, No. 1020: Structural and Quantitative Studies on the Normal C3H and Lurcher Mutant Mouse, By K.W.T. Caddy and T.J. Biscoe. Pp.167-201. + plates 1-15. UK £5.80; Overseas £6.10. (London: The Royal Society, 1979.) (1510

National Coal Board. Coal Research Establishment— Annual Report, April 1978 to March 1979. Pp.32 (Stoke Orchard, Cheltenhjam: National Coal Board, Coal Research Establishment, 1979.) (1610

Nature Conservancy Council. Nature Conservation in the Marine Environment. Pp.65. Published jointly with the Natural Environment Research Council. (Attingham Park, Shrewsbury: NCC, Interpretative Branch, 1979.) £2.50 (1610

Public Health Laboratory Service. Monograph Series, No. 11: The Vibrios. By A.L. Furniss, J.V. Lee and T.J. Donovan. Pp.viii + 58. (London: HMSO, 1978.) £2 net. (1810

Control and Suppression of Pressure Surges in Pipelines and Tunnels. By A.R.D. Thorley and K.J. Enever. (CIRIA Report 84.) Pp.112. (London: Construction Industry Research and Information Association, 6 Storey's Gate, SW1, 1979.) £40 (CIRIA Members £10.) (1810

Arms Control and Disarmamenr: Developments in the International Negotiations, No. 1, August 1979. Pp.28. (London: Arms Control and Disarmament Research Unit, Foreign and Commonwealth Office, Downing Street (East), 1979.) (1910

British Nutrition Foundation. Annual Report, 1978-1979. Pp.26. (London: British Nutrition Foundation, 15 Belgrave Square, SW1, 1979.) (1910

Nature Conservation in the Marine Environment. (Report of the NCC/NERC Joint Working Party on Marine Wildlife Conservation.) Pp.65. (Shrewsbury: Interpretative Branch, Nature Conservancy Council, Attingham park, 1979.) £2.50. (2210

Health and Safety Commission. Dangerous Pathogens: Draft Regulations and Draft Guidance Notes. (Consultative Document.) Pp.11. (London: HSE General/Enquiry Point, Baynards House, 1 Chepstow Place, W2, 1979.) 50p. (2210

Health and Safety Commission. Safety Helmets on Construction Sites: Recommendations of the Construction Industry Advisory Committee on Their Provision and Use. (Discussion Document). Pp.11. (London: HSE General Enquiry Point, Baynards House, 1 Chepstow Place, W1, 1979.) 50p net. (2310

Department of the Environment: Central Directorate on Environmental Pollution. Chlorofluorocarbons and Their Effect on Stratospheric Ozone, (Second Report). (Pollution Paper No.15.) Pp.xii + 236. (London: HMSO, 1979.) £4.75 net. (2410

Thames Water. Annual Report and Accounts for the year ended 31st March 1979. Pp.46 + 28. (London: Thames Water Authority, New River Head, Rosebery Avenue, EC1, 1979.)

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APPOINTMENTS VACANT

HOUGHTON POULTRY
RESEARCH STATION
SCIENTIFIC OFFICER
HISTOLOGY

A vacancy exists in the Histology Section for a scientific officer to assist with the provision of routine histological services and the performance and development of specialised techniques.

Applicants must have a degree, HNC or equivalent qualification with several years' experience in histology.

The appointment will be to the Scientific Officer grade (£3,289 × 12 to £5,085) which will be revised to (£3,591 × 10 to £5,486) with effect from 1st January 1980 with a starting salary depending upon qualifications and experience. Non-contributory superannuation scheme.

Further particulars and application forms from the Station Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA. Closing date for application is 20th December 1979. 3852(A)

THE UNIVERSITY OF ADELAIDE
South Australia
SENIOR RESEARCH OFFICER

to work on a project on transformation of human epithelial cells by Herpes simplex virus. Details may be obtained from Dr. M A Stanley in the University. Salary $A15,786 × 7 — $20,738 (N.H. & M.R.C.), available for up to 3 years.

Applications in duplicate, giving personal and academic particulars plus names and addresses of two or three referees to reach the Registrar of the University, G.P.O., Box 498, Adelaide, South Australia 5001 by *21 December 1979*. *Senior Research Officer* for 3 years to work on organization and control of human collagen genes. Salary $A15,786 × 7 — $20,738 (N.H. & M.R.C.). For details write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or The Registrar of the University, with whom applications close as soon as possible. 3855(A)

UNIVERSITY OF LONDON
Institute of Psychiatry
DEPARTMENT OF NEUROLOGY
RESEARCH ASSISTANT

Applications are requested from recently qualified graduates in pharmacology, biochemistry or related disciplines for a project investigating the effects of long-term neuroleptic administration on cerebral dopamine mechanisms.

The post is tenable for 2 years, renewable annually.

Salary on Research Scale 1B. — £3,775 to £4,333 plus £740 London weighting.

Application forms can be obtained from: The Secretary, Institute of Psychiatry, De Crespigny Park, London SE5 8AF. 3848(A)

UNIVERSITY OF STIRLING
DEPARTMENT OF EARTH & ENVIRONMENTAL SCIENCE
RESEARCH ASSISTANT

A Research Assistant is required from 1 March 1980 for a period of 2 years, to work on an NERC-funded research programme monitoring temperature regimes in the inter-tidal mudflats of the Forth Estuary. Applications are invited from graduates with good honours degrees in Environmental Science, Biology, or Geography who have at least 2 years postgraduate research experience. Preference will be given to applicants with practical experience in environmental monitoring and/or environmental data handling. Starting salary will be on the first point of the Research Assistant 1B Scale, at £3,775.

Applications, giving the names of 2 referees should be submitted by 21 December 1979, to The University Secretary (N), University of Stirling, Stirling FK9 4LA, from whom further particulars may be obtained. 3839(A)

Assistant Fisheries Officer

£6,003 — £6,426 p.a.

This post is in the Authority's Amenity, Fisheries and Recreation Department based in Central Leeds.

The successful applicant will be responsible to the Fisheries Officer and will assist him in relation to fisheries management advice and fisheries policies and especially in dealing with the issue of statutory consents under Fisheries legislation. Particularly he/she will be responsible for the collection, collation and assessment of scientific reports and information relating to fisheries functions and the production of fisheries reports for the Authority's committees and publications.

Applicants must possess a degree in a Biological Science and have at least two years post graduate experience. Experience of information retrieval and statistics would be an advantage.

Removal expenses and disturbance allowances will be available in approved cases and appropriate car user allowance is payable.

Application forms may be obtained from the Regional Personnel Officer, Yorkshire Water Authority, 67 Albion Street, Leeds, LS1 5AA (Tel. Leeds 448201 Ext. 269) to whom they should be returned by 10 December 1979 quoting Ref. RN 719.

3833(A)

Yorkshire Water

INSTITUTE OF TERRESTRIAL ECOLOGY
SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

The Institute has a vacancy for a Scientific Officer/Higher Scientific Officer within a team engaged in genetic evaluation and selection of young conifers and broad-leaved trees. Current work involves field and laboratory studies on competition and frost hardiness and will include the collection, propagation and evaluation of broad-leaved trees. There will be an opportunity to contribute to new lines of research on physiological genetics of trees. The post will be based at Bush Estate, Penicuik, near Edinburgh.

Qualifications: Candidates should have a good honours degree or equivalent in applied plant science or forestry and preferably some practical experience. Candidates for Higher Scientific Officer should have at least two years post graduate experience for those with an Honours degree or five years experience for those with HNC/HND.

Salary and conditions of service: The salary will be within the following scales: SO £3,289 to £5,085; £3,591 to £5,486 revised rate from 1.1.80. HSO £4,727 to £6,211; £5,097 to £6,737 revised rate from 1.1.80.

Up to 22 days annual leave plus 10½ days public and privilege holidays. Starting pay according to age, qualifications and experience. There is a non-contributory superannuation scheme.

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from: Establishments Section, Institute of Terrestrial Ecology, 68 Hills Road, Cambridge, CB2 1LA. Telephone: (0223) 69745.

Closing date for completed applications is 14 December, 1979. Reference 124.
Natural Environment Research Council. 3814(A)

THE POLYTECHNIC OF WALES Department of Science, Anaerobi Digestion Research Technician (Salar £3,894) required to join in a projec investigating factors affecting methan production during the anaerobic digestio of animal wastes. Applicants should have degree or equivalent in a field whic includes biochemistry and microbiology and a good working knowledge o techniques in analytical chemistry an microbiology. The post is of three years duration and is funded by the SRC. Furthe particulars from: Asst. Director (Staffing) The Polytechnic of Wales, Pontypridd Mid Glamorgan, CF37 1DL. Telephone Pontypridd (0443) 405133, Ext. 202 Closing date: 17 December 1979. 3842(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
TECHNICIAN POSTS IN FOOD SCIENCE

Grade 4 in food chemistry laboratorie Experienced in chemistry teachin laboratories essential. C & G Scien Laboratory Technicians' Certificate equivalent desirable. Salary on scale £3,4 to £3,950 p.a. (under review) plus Lond weighting £780 p.a.

Trainee to assist with classes in foo science. Day release available for approved qualification. GCE 'O' level chemistry or physics or biology require Salary (under review) depending on age scale £1,834 to £2,553 p.a. plus Lond weighting of £780 p.a.

Applications, giving details education, age, and other experience, a names of two referees, to the Colle Secretary, Queen Elizabeth Colleg Campden Hill Road, London W8 7AH. 3832(A)

PROGRAM DIRECTOR OF PHYSICAL AND CHEMICAL OCEANOGRAPHIC DATA FACILITY AT SCRIPPS INSTITUTION OF OCEANOGRAPHY, OCEAN RESEARCH DIVISION

The Facility constitutes one of the most advanced groups specializing in the collection and processing of physical and chemical measurements in the ocean. Programs carried out encompass Institutional, National and International oceanographic programs. The Program Director of the Facility is responsible for the guidance of this group as well as for directing the development and introduction of new oceanographic techniques, instrumentation, and data processing procedures. Participation in the scientific endeavors of the various programs is encouraged. At present the Facility employs forty people. Academic Administrator salary range $30,000 to $42,000. For further information, address Search Committee, P.A.C.O.D.F. C/O Chairman, Ocean Research Division, A-020, Scripps Institution of Oceanography, La Jolla, CA 92093. The University of California is an Equal Opportunity/Affirmative Action Employer. W572(A)

CANCER RESEARCH UNIT, University of Western Ontario, London, Ontario. Positions are available for staff at the Assistant or Associate Professor level (non-tenure track) and for postdoctoral fellows and graduate students. Research is focussed on the areas of cellular and viral DNA replication, nucleic acid metabolism in eukaryotic cells, mRNA cloning, and mechanisms of cell transformation.

The unit is well funded by the National Cancer Institute of Canada and is affiliated with the Department of Biochemistry and the Department of Microbiology and Immunology in the Medical School of the University.

Candidates for a staff position should submit a curriculum vitae, statement of research interests, and the names of three referees to Dr. David T. Denhardt, c/o The Administrative Secretary, Cancer Research Unit, University of Western Ontario, London, Ontario N6A 5B7, Canada. Students should apply for admission through the above mentioned Departments. W544(A)

STANFORD UNIVERSITY

GEOCHEMIST —PETROLOGIST

The Department of Geology in the School of Earth Sciences invites applications for a faculty position beginning September, 1980. Areas of interest include (but are not limited to) geochemical processes in the crust and mantle of the earth including isotope geochemistry, thermodynamics, kinetics, element distribution and phase equilibria studies. Teaching responsibilities might include graduate and undergraduate courses in geochemistry and igneous petrology. Level of appointment is open, but we are particularly interested in a creative young person, with a well-established record in research.

Applications should include (1) a resume of education, professional experience, and published research; (2) a brief statement of plans for future research, and (3) the names of 4 referees. Send to: Prof. J.G. Liou, Department of Geology, Stanford University, Stanford, CA 94305, by December 1st, 1979. Stanford University is an equal-opportunity/affirmative-action employer. W329(A)

UNIVERSITY OF WÜRZBURG

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY

German Federal Republic

Applications are invited for POSTDOCTORAL FELLOW POSITIONS (Wissenschaftliche Mitarbeiter, BAT IIa)

for two years or longer, which will become vacant on March 1, 1980. The salary will be about DM42,000 per annum. German citizenship is not a prerequisite. Candidates must have a PhD in Biochemistry and an active research interest in Molecular Endocrinology and Isolation and Characterisation of Membrane Proteins.

Applications with a curriculum vitae, qualifications and grades and the names of two referees should be sent until January 1, 1980 to Ernst J M Helmreich, Professor and Chairman, 8700 Würzburg, FRG, Koellikerstrasse 2, Tel: 0931/31745 und 46. W562(A)

QUEEN ELIZABETH COLLEGE

Kensington

(University of London)

ELECTRON MICROSCOPE TECHNICIAN, Grade 5

required in the Microbiology Department to run a service used by the Departments of Microbiology, Physiology and Biochemistry.

Equipment is an AEI EM6B electron microscope and a JEOL 25/S11 scanning electron microscope is to be installed shortly. The technician will be expected to operate and maintain the microscope and advise staff and students in the techniques, and will need the appropriate qualifications. Salary on scale £3,700 to £4,320 p.a. (under review) plus London weighting £780 p.a.

Applications giving a curriculum vitae and names of two referees to the College Secretary, Queen Elizabeth College, Campden Hill Road, London, W8 7AH. 3830(A)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF PSYCHOLOGY

RESEARCH ASSISTANT: ANIMAL BEHAVIOUR

Applications are invited for a post of research assistant to work with A Whiten and J Graves on a continuing SRC Project "The Development of Parent-Offspring Relationships in the Herring Gull". Applicants should have a relevant first degree, and preference will be given to those with experience of behavioural, ecological and/or ornithological study. The successful applicant will conduct observational and experimental research on this topic on the Isle of May and Holy Isle, off Arran, and subsequently collaborate in analysis and publication.

The appointment must begin in March 1980 or earlier, and may last for up to 2½ years. Starting salary scale within range £3,775 to £4,910 according to age and qualifications, plus FSSU/USS.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by 14th December 1979 with the Establishments Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained. 3840(A)

CSIRO AUSTRALIA

Post Doctoral Research Fellow (2 Positions)

Fuel Geoscience Unit

North Ryde NSW

CSIRO has a broad charter of research into primary and secondary industry areas. The Organization has approximately 7000 employees — 2400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

GENERAL: The Fuel Geoscience Unit conducts research relevant to exploration for the fossil fuels — coal, petroleum, natural gas and oil shale — and to their assessment and extraction.

FIELD: Coal Utilisation — Position number 604/168

DUTIES: To initiate and conduct, within a team, fundamental research related to conversion of coals to liquid fuels, with special emphasis on coal hydrogenation. The appointee will be required to use both microscopic and chemical techniques to study the properties of coals and of products obtained from coals after treatment.

QUALIFICATIONS: A PhD or equivalent qualifications together with experience in the field of either coal chemistry or coal petrology.

FIELD: Coal Mine Geology — Position number 604/169

DUTIES: To initiate and conduct research aimed at generating information relevant to coal mining prior to the construction of mine openings. The appointee will be, or should become, familiar with geological factors of importance in open and underground coal mining and will be required to apply some of the tehniques of sedimentary petrology (including microscopy) and engineering geology to studies of coal seams, overburdens and inter-seam sediments.

QUALIFICATIONS: A PhD or equivalent qualifications in a relevant area of geology. Experience appropriate to the duties above, especially concerning the significance of the physical and chemical properties of coals and rocks to mining practice would be an advantage.

SALARY: Appointment for both positions will be within the range of Research Scientist/Senior Research Scientist: $A15,916 — $A23,122 p.a.

TENURE: Three years with the possibility of extension.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the appropriate reference number(s) should reach The Personnel Officer, Australian Scientific Liaison Office, 10-16 Maltravers St, London WC2R 3EH by 28th December, 1979. Applications in USA and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036. 3834(A)

University of Basle

Faculty of Science

In connection with the reorganisation of the Department of Zoology applications are invited for the position of

PROFESSOR (full professor)

The position will become available at the end of 1980.

Experience is desired in one of the following fields: ecological ethology, morphology including electron microscopy.

Duties include general teaching in zoology and collaboration in research and teaching with special emphasis on ecological aspects.

Applications stating publications, teaching experience, curriculum vitae etc. should be submitted until January 1980.

Address: Dekanat der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel, Klingelbergstrasse 70, CH-4056 Basel.

No further application is required for those who already have applied for another, previously advertised position in the Department of Zoology. Complementary material may be submitted, however. W566(A)

The Wellcome Research Laboratories Beckenham

Wellcome

Biochemist £6,000-£11,000*

A Biochemist is required in the Department of Parasitology to join a team studying electron transport systems and related topics in parasitic protozoa and helminths, with the aim of more effective chemotherapy of the diseases caused by these organisms.

Candidates should be familiar with those techniques used to study electron transport processes in mitochondrial preparations, particularly spectrophotometry and physicochemical methods. A PhD in biochemistry is essential and the successful candidate will have either recently completed the degree or have a few years' post-doctoral experience in this area of biochemistry. *Salary, dependent on age and experience, will be within the range £6,000 to £11,000.

The Laboratories are situated in pleasant parkland surroundings within easy reach of Central London and conditions of employment are excellent and include subsidised canteen, pension scheme, 4 weeks' holiday, relocation expenses where appropriate and sports and social facilities.

For an application form please write, giving brief details of background and experience, to

the Personnel Manager,
Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent BR3 3BS.

1979

3827(A)

THE DEPARTMENT OF BIOLOGICAL SCIENCES OF HUNTER COLLEGE CUNY

welcomes applicants for a tenure-track faculty position.
Applicants with expertise in any of the following areas will be favored: ecology, physiological ecology (plant or animal), population biology/evolution, and plant physiology/virology. The candidate should have post-doctoral experience and is expected to establish a research program in addition to teaching duties.
Send curriculum vitae, names of 3 references & a statement of career goals to:

DR C CECCARINI
CHAIRPERSON, SEARCH COMMITTEE
DEPT OF BIOLOGICAL SCIENCES
HUNTER COLLEGE OF CUNY
695 PARK AVENUE
NEW YORK, NY 10021

An equal opportunity/affirmative action employer. **W573(A)**

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

RESEARCH ASSISTANT

The University of Leicester is embarking on a project — supported by the Royal Observatory, Edinburgh — to develop focal plane instrumentation for the 3.4m United Kingdom Infra Red Telescope (UKIRT) in Hawaii. A post exists for a research assistant to join the project, firstly to investigate applications of multi-element CID imaging detectors in infra-red astronomy; secondly to develop instrumentation for UKIRT using these devices, and thirdly to take part in astronomical observations using UKIRT.

Salary will be within the range £4,333 to £5,488 depending upon qualification and experience. Superannuation is under USS conditions.

Applications, together with the names and addresses of two referees, and a curriculum vitae, should be sent to Professor K A Pounds, Department of Physics, University of Leicester, LE1 7RH by December 17th 1979. Further particulars about the project are available on request. 3843(A)

UNIVERSITY COLLEGE LONDON

SCIENCE RESEARCH COUNCIL COLLABORATIVE PROJECT ON

ASTRONOMICAL SPECTRA

The Science Research Council has established a Collaborative Computational Project concerned with the Analysis of Astronomical Spectra. Research workers at the following institutions will be participating in the project: Universities of Belfast, Cambridge, Cardiff, London, Oxford and St. Andrews, the Royal Greenwich Observatory and the Royal Observatory Edinburgh.

The work will be concerned with stellar atmosphere theory (maintaining a library of well-documented computer programs, developing new techniques, maintaining collaboration with research groups at home and abroad, and collaboration with groups studying relevant physical processes) and with the interpretation of other astronomical spectra. A Research Assistant will be appointed to work on the Project in the Department of Physics and Astronomy, University College London, and will be expected to maintain close contact with the Theory and Computation Division at the Science Research Council's Daresbury Laboratory and with the other participating institutions. Applicants should have a PhD or equivalent qualification, extensive experience of computational work, and preferably expert knowledge of stellar atmosphere theory or other aspects of radiative transfer theory.

The starting salary is in the range £4,333 to £5,199 p.a. plus £740 p.a. London Allowance. Applications should be addressed to Professor M J Seaton FRS, Dept of Physics and Astronomy, University College London, Gower St, London WC1E 6BT, and should be received by 1 January 1980. 3838(A)

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McGILL UNIVERSITY

POSTDOCTORAL POSITION: CELL BIOLOGY

Applications are invited for a postdoctoral position for 2 years. The post is for research in the regulation of DNA replication and functions of tumor antigens in mammalian cells. It is available April 1, 1980. Previous experience in cell culture is required and training in molecular biology, animal virology or somatic cell genetics would be helpful. The research group includes other postdoctoral fellows and graduate students. Opportunities are available for interaction with other groups interested in nucleic acid research.

Applications with curriculum vitae and names of reference to: Dr Roger Hand, McGill Cancer Centre, 3655 Drummond, Montreal, Que. H3G 1Y6. W575(A)

UNIVERSITY OF RHODE ISLAND

Narragansett, Rhode Island 02882

MARINE RESEARCH ASSOCIATE II or III

Responsible for studies in phytoplankton and zooplankton production, species composition and abundance and other aspects of plankton ecology in enclosed ecosystems affected by low level chronic pollutants or other perturbations; supervise technical personnel, sampling program, data acquisition and analysis, scholarly writing and independent research. PhD, and experience in plankton required.

Submit résumé and the names of three references by December 31, 1979 to: Dr Candace Oviatt, MERL Graduate School of Oceanography. An Affirmative Action/Equal Opportunity Employer. W571(A)

IMPERIAL COLLEGE (University of London) ENHANCED OIL RECOVERY RESEARCH

Applications are invited for post-doctoral research posts (SRC sponsored) tenable for up to three years for the study of: 1) the behaviour of emulsions in reservoir rocks, 2) capillary effects in the enhanced recovery of oil from reservoir rocks.

The work is of fundamental importance to oil recovery processes and requires an appreciation of fluid-surface interfacial phenomena and of porous media.

The usual SRC conditions apply, with a starting salary within the range of research and analogous staff, grade 1A, £4,333 to £7,521 (under review) plus £740 London Allowance, and the posts carry USS benefits. Initial placing dependent on age, qualifications and experience.

Enquiries and applications (giving curriculum vitae and the names of two referees) to Dr R A Dawe, Mineral Resources Engineering Department, Imperial College, London SW7 2BP.

A number of studentships for study to PhD are also available. 3837(A)

CANBERRA COLLEGE OF ADVANCED EDUCATION

SCHOOL OF APPLIED SCIENCE

COLLEGE FELLOW IN LIFE SCIENCES (Ref. No. 79/2177)

The College Council has reviewed recently the structure of the School of Applied Science and has decided that the importance attaching to the teaching of the Life Sciences in the College is such as to justify the establishment of a position of College Fellow at professorial level.

Applications are invited from persons with academic and professional qualifications and experience appropriate to such a senior academic post in a School which is concerned with the teaching of applied science in its broadest sense. The School of Applied Science has developed degree and diploma programmes of an inter-disciplinary nature in Land Science Park Management, Resource Management, Natural Resources, Ecology Human Biology, Medical Technology Nursing Science, Community Health Nursing, Applied Geology, Cartography Physics and Electronics, Material Conservation, Resource Management Recreation Planning, and Electronics Planned development for the future includes the establishment of basic and post-basic courses in Nursing and in other health-related fields. The College has an established reputation in the community for the variety and strength of its vocational course programmes.

The appointee will be required to provide academic leadership in the broad area of the Life Sciences within the School, to play a significant role in the academic development of the School as a whole, and to assist in its day-to-day administration. The appointee will also be required to carry some teaching duties in his/her own special area of expertise.

Salary: $A34,119 per annum. Assistance with housing will be provided for a person moving to Canberra to take up appointment. Fares for the appointee and family and reasonable removal costs will be paid.

Applications should give personal details, particulars of qualifications, experience, previous appointments, present position, telephone number during business hours and the names and addresses of three referees. Applications quoting reference number are required by 15 January 1980 and should be addressed to: The Registrar, Canberra College of Advanced Education, PO Box 1 Belconnen Act 2616, Australia, and a copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square London WC1H 0PF. 3820(A)

Experimental Officer

A vacancy exists for a person with a first degree in Biology, Agriculture or related subject to lead a small team which investigates the potential of new seed treatments for the control of cereal diseases. The job holder, who will respond to the Senior Cereal Pathologist, should have the ability to plan and conduct experimental programmes in the glasshouse and field, and have a good general knowledge of plant pathology and chemistry.

Applicants should have some experience of crop protection research, and previous work on the treatment or testing of seeds would be particularly valuable.

Jealott's Hill is the chemical and biological research centre for the crop protection interest of ICI. It is situated in open country 30 miles west of London, between Bracknell and Maidenhead. We offer competitive salaries and fringe benefits including membership of the ICI Profit Sharing and Pension Fund. Coaches run between Jealott's Hill, Bracknell and Maidenhead.

If you are interested in this vacancy please write or telephone for an application to:

Mr S R Stephenson, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell. Telephone 24701

ICI Plant Protection Division

3824(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
COURTAULD INSTITUTE OF BIOCHEMISTRY

A POSTDOCTORAL and also a GRADUATE RESEARCH ASSISTANT

e required from 2nd January 1980 to join group interested in the mechanism of tion of the Carcinogenic N-introso mpounds.

Candidates should have degree alifications in Biochemistry (or possibly emistry) and be interested in developing immunological approach dioimmunoassay and immunocytology) the study of carcinogen-nucleic acid eraction. These positions are funded by Cancer Research Campaign for a fixed riod.

Starting salaries, according to age, alifications and experience, £5,073 to 228 per annum (Postdoctoral) and 515 to £5,361 per annum (Graduate) lusive of London Allowance.

Applications together with names and dresses of 2 referees to Dr P F Swann, urtuld Institute of Biochemistry, The ddlesex Hospital Medical School, ndon. W1P 5PR. 3847(A)

THE UNIVERSITY OF AUCKLAND
New Zealand

BIOCHEMISTRY
Lectureship

Applicants should have a first degree in biochemistry or chemistry and suitable postgraduate experience in biochemistry. Preference will be given to candidates who have completed a period of postdoctoral research. The successful applicant will be required to teach biochemistry to undergraduates, to give suitable advanced courses at the masters level and to supervise laboratory work by research students. Commencing salary will be determined in accordance with qualifications and experience within the range NZ$13,722 to $16,861 per annum.

Conditions of appointment and Method of Application are available from the Academic Appointments Office, University of Auckland, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications, in accordance with 'Method of Application' should be forwarded as soon as possible but not later than 29 February, 1980. 3829(A)

Public Service Canada / Fonction publique Canada

Open to both men and women

Fisheries and Oceans Canada
Marine Ecology Laboratory
Dartmouth, Nova Scotia

RESEARCH SCIENTISTS

Salary: Up to $36,428

The Bedford Institute of Oceanography located in Nova Scotia, Canada, one of the world's largest Oceanographic Institutes, has need for Research Scientists in the following specialities:

Microbiologist (119-291-011)
Ref. No.: 79-PSCH-DFO-SE-6073

Conducts research on micro-organisms in the marine environment with emphasis on nutrition, growth, bio-energetics, and on the role of micro-organisms in the tropic dynamics of the marine ecosystem.

Qualifications
PhD in microbiology, oceanography or similar field. Research experience in ecology or marine micro-organisms; ability to conduct research at sea and to plan and conduct studies of structure and function of the marine phytoplankton community.

Physical Oceanographer (119-291-002)
Ref.No.: 79-PSCH-DFO-SE-6072

Plans and conducts research on the physical processes which determine biological productivity with emphasis on continental shelfs. The research involves participation in work at sea as well as extensive use of numerial simulation and analysis techniques.

Qualifications
PhD or equivalent research experience in physical oceanography, physics, applied mathematics or related field. Research experience and demonstrated capacity to make relevant original contributions.

Zooplanktologist (119-261-002)
Ref.No.: 79-PSCH-DFO-SE-6074

Conducts research on marine secondary production processes among representative groups in the pelagic community, with the objective of developing biochemical indices of resource transfer and energy utilization for use in food chain modelling and production studies.

Qualifications
PhD or equivalent research experience in marine biology, oceanography or biochemistry with a sound general knowledge of ecology and invertebrate zoology especially marine zooplankton. Ability to conduct research at sea.

Language Requirements
Knowledge of English is essential for all these positions.

"Additional job information is available by writing to the address below;
Toute information relative à ce concours est disponible en français et peut être obtenue en écrivant à l'adresse suivante".

How to apply
Send your application form and/or résumé to:
Mrs E Cruickshank (902) 426-2169
Public Service Commission of Canada
4th Floor, 5161 George Street
Halifax, Nova Scotia B3J 1M8
Closing Date: December 28, 1979 W565(A)

Please quote the applicable reference number at all times.

RESEARCH SCIENTIST

An opportunity to gain valuable experience in the design and synthesis of fluorinated bioactive agents.

. . . with one of the world's most highly regarded pharmaceutical companies. This two-year postdoctoral appointment at our Rahway, New Jersey laboratories requires a highly motivated person with a PhD in chemistry to participate in research in the design and synthesis of fluorinated bioactive molecules including agents to interfere with the biosynthesis, release, degradation, or action of neurotransmitters. The appointment carries a first year stipend of $18,000.

To explore, this opportunity, please send resume to Mrs. S. R. Jenkins,

MERCK & CO., INC.
P.O. Box 2000, Rahway, NJ 07065

An equal opportunity employer m/f
W569(A)

UMIST
Department of Ophthalmic Optics
RESEARCH STUDENTSHIP

A suitably qualified graduate is needed to carry out research on the interaction of the eye with optical instruments, particularly sighting telescopes, and to investigate the effect of such factors as ocular accommodation and aberration on the overall performance achieved. The stipend is at normal Research Council rates and the project will initially run for a period of one year; the successful candidate will register for an MSc degree.

Further details may be obtained from Dr W N Charman, Ophthalmic Optics, UMIST, PO Box 88, Manchester M60 1QD. 3836(F)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

LECTURER: MOLECULAR GENETICS

Applications are invited for the post of Lecturer, in Molecular Genetics, in The School of Biological Sciences. Applicants should have research interests in molecular genetics and experience in modern methods of gene manipulation and DNA biochemistry. The salary will be within the scale £4,333 to £8,992 (under review) with superannuation benefits.

Application forms and further particulars can be obtained from the Assistant Secretary of Science, Science Office (E), Sussex House, University of Sussex, Falmer, Brighton BN1 9RH, to whom applications (8 copies) should be sent by 14th January 1980. 3822(A)

UNIVERSITY COLLEGE OF NORTH WALES
Bangor

RESEARCH OFFICER IN ORGANIC CHEMISTRY

Applications are invited for the above one year SRC-supported postdoctoral post to work on strain-accelerated elimination reaction, in collaboration with Professor C J M Stirling, to whom informal enquiries may be made. (Bangor 51151, Extension 223.)

Salary will be in the range £4,333 to £4,910 per annum (under review).

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of two referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2UW as soon as possible. 3845(A)

QUEEN MARY COLLEGE
(University of London)

GEOLOGICAL SCIENCES DEPARTMENT

Postgraduate or postdoctoral
RESEARCH ASSISTANT

required for one year to assist work on the influence of fabrics on the transference of water-soluble ions through soils, a project supported by I.C.I.

Candidates should have a degree in geology, chemistry, geochemistry, geotechnology or physics.

Salary, according to qualifications and experience, in range (under review) £3,775 to £4,910 p.a., plus £740 London Allowance.

Please apply, giving details of age, qualifications, experience and two referees, to The Secretary, (N) Queen Mary College, Mile End Road, London E1 4NS, by 14 December. 3849(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF COMMUNITY MEDICINE

RESEARCH ASSISTANT

Applications are invited for the post of graduate research assistant to work on Epidemiological studies in the fields of diabetes and other chronic diseases. The post is tenable for three years and applicants should have a higher degree or equivalent experience in epidemiology, demography or medical statistics. Starting salary up to £5,940 per annum according to age, qualifications and experience.

For further details contact: Dr J.H. Fuller, Department of Community Medicine, Horace Joules Hall, Central Middlesex Hospital, London NW10 7NS, to whom applications should be sent by 21 December, 1979. 3831(A)

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nature
London
3 Dyers Buildings
London EC1N 2NR
01-831 6901
New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

UWIST
University of Wales
THE WELSH SCHOOL OF PHARMACY

RESEARCH ASSOCIATE

To develop a method for measuring Plasma levels of Anti Diabetic Sutphonylureas.

Salary: within range 1A/£5,199

Requests (quoting Ref N) for details and application forms to Personnel Section (academic), UWIST, Cardiff CF1 3NU.

Preliminary enquiries may be made of Doctor P Takla, (Telephone 0222-42522).
Closing date: 20th December 1979 3854(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF CARDIOVASCULAR MEDICINE

Applications are invited for the post of

RESEARCH STUDENT

from persons with a good honours degree in Biochemistry, Physiology or related subjects to study aspects of the contractile and regulatory proteins of the human myocardium in normal and pathological conditions. The post which is supported by the British Heart Foundation is available from October 1979 and is funded for 3 years.

Applicants will be expected to register for the degree of PhD.

Further details can be obtained from Dr Peter Cummins, Department of Cardiovascular Medicine, Clinical Research Block, Queen Elizabeth Hospital, University of Birmingham, Birmingham B15 2TH, to whom applications, together with curriculum vitae and names of two referees should be returned not later than Friday 14th December. 3826(A)

TECHNICIAN — BIOLOGY
£3400 to £3800
THE CITY OF LONDON POLYTECHNIC

invites applications for a vacant post of Technician 2B in the Department of Biological Sciences. This Department is located at Calcutta House, Old Castle Street, London E1 close to Aldgate and Aldgate East Underground stations.

The successful candidate will assist the Biochemistry Technician Grade 6 in servicing practical classes and carry out other general duties.

Normal background experience required is 3-5 years (including training period) and minimum qualifications will be ONC OND, 2 'A' levels, or C & G.

Good conditions of service include 37 hour week, 18 days annual leave (rising to 21 with service), sick pay, contributory pension scheme. The Polytechnic offers positive encouragement to staff wishing to study for relevant qualifications.

Salary on a scale from £3462 to £3864 which will increase in April 1980.

For an application form and job description please write to the Staff Records Officer, City of London Polytechnic, 117 Houndsditch, London EC3A 7BU. Please quote reference 79/144. 3816(A)

NORTHWESTERN UNIVERSITY
Geological Sciences

Seizing an opportunity to strengthen the Department, the Department plans to recruit five faculty over the next two years. Two or three of the following positions are to be filled in 1980:

SENIOR PROFESSOR
(to become William Deering Professor on retirement of L L Sloss in 1981) to provide leadership in broad Crustal Evolution Program; global stratigraphy and sedimentation or some analogous fields.

GEOCHEMIST
(probably Assistant or Associate Professor) with strong kinetics-thermodynamics background and interests in chemical and physical interactions among surface waters, sediments, and atmosphere.

Solid Earth **GEOPHYSICIST** (Assistant Professor): theoretical methodology would be most compatible with general departmental research, but search not limited to theorists.

Send nominations, applications, and inquiries to E H Timothy Whitten, Chairman, Department of Geological Sciences, Northwestern University, Evanston, Illinois 60201, USA.

An Equal-Opportunity/Affirmative-Action Employer. W576(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF PATHOLOGY
Applications are invited for the post of LECTURER (CLINICAL OR NON-CLINICAL)

with duties in the Section of Immunopathology.

Applicants should have wide experience in immunopathology, with particular interests in humoral mechanisms and human immunopathology.

Duties will involve participation in the diagnostic service, teaching (intercalated-degree medical students and supervision of postgraduates), and research.

Informal enquiries should be made to Professor J. Swanson Beck (telephone 0382 60111 ext. 2169).

Further particulars can be obtained from The Secretary, The University, Dundee DD1 4HN to whom applications (eight copies) giving details of qualifications, experience and names of three professional referees should be submitted by 22nd December 1979. Please quote Reference EST/97/79]. 3835(A)

UNIVERSITY OF EDINBURGH
CENTRE FOR REPRODUCTIVE BIOLOGY
DEPARTMENT OBSTETRICS AND GYNAECOLOGY
LECTURER (NON-CLINICAL)

Applications are invited for the above post in the Department of Obstetrics and Gynaecology. Preference will be given to those who have experience in the biochemistry of reproduction. The successful candidate will be expected to conduct research in reproductive endocrinology and to participate in the teaching of undergraduate and supervision of postgraduate students. There are excellent facilities for collaborative work with clinicians in the Royal Infirmary and scientists in the MRC Unit of Reproductive Biology.

Salary on the scale £4,333 to £8,992 per annum. USS Superannuation.

Enquiries may be addressed to Professor D T Baird, Department of Obstetrics and Gynaecology, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh, (031 229 2575)

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (8 copies) including curriculum vitae and the names and addresses of three referees should be lodged not later than 21st December 1979. Please quote Reference 1085. 3846(A)

Food Scientists

London Area

Our client is a major British food company, with a diversified range of high quality products. A major further diversification programme is now under way and this has led to a requirement for the following key managers.

Fermentation Manager

– to lead a team of microbiologists working on a range of fermentation processes.

Project Manager
Food Products

– to identify and develop new food ingredients with the company's expansion criteria.

In both cases, a Ph.D. in a biological or chemical science would be desirable. Experience in a similar or related field in a scientific managerial role will be necessary.

Generous salaries and fringe benefits would be offered.

Candidates (male or female) should submit full résumés, which will be treated in full confidence, to the consultants handling the appointment.

Anthony Whitmee & Associates,
86 WIMPOLE STREET, LONDON W1M 7DB

SCIENTIST
(PhD or equivalent)

with biomedical background and at least five years' research experience, required for challenging post in an organisation concerned with promoting alternatives to laboratory animals. Salary according to age and experience.

For further details apply to FRAME (Fund for the Replacement of Animals in Medical Experiments) 312a Worple Road London SW20 8QU. 3853(A)

UNIVERSITY
OF ALASKA
MICROBIAL ECOLOGIST

The Institute of Marine Science at the University of Alaska will fill at the rank of assistant professor a tenure-track position in one of the several areas related to marine microbial processes.

The successful applicant will provide evidence of a strong background in fundamentals and of experience related to natural processes. Participation in interdisciplinary studies is encouraged. A letter of application, curriculum vitae, and the names of three references should be sent to Dr Vera Alexander, Acting Director, Institute of Marine Science, University of Alaska, Fairbanks.

The University of Alaska is an Affirmative Action/Equal Opportunity Employer. Closing date; January 6, 1980. W577(A)

UNIVERSITY
OF LONDON
KING'S COLLEGE

BIOCHEMIST OR
PHARMACOLOGIST

Research Assistant required at postgraduate or postdoctoral level to work on mechanism of action of nicotine-like drugs. Appointment at appropriate position on University scale.

Knowledge of membrane or lipid biochemistry an advantage.

Please apply in writing to Dr J M Littleton, Department of Pharmacology, King's College, London WC2R 2LS. 3817(A)

UNIVERSITY
OF OXFORD
MRC MOLECULAR
HAEMATOLOGY UNIT

Applications are invited for a
POST-DOCTORAL
RESEARCH ASSOCIATE

to join a group working on human haemoglobin gene expression.

Candidates preferably should have had experience in the use of re-combinant DNA techniques or chromatin transcription systems. Salary in the range £3,775 to £6,355 (under review), according to age and experience.

Applications, together with curriculum vitae and the names of three referees, should reach Professor D J Weatherall, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, by: 31.12.79. 3821(A)

The Department of Pathology at the University of Pittsburgh School of Medicine wishes to recruit a member for its Division of Experimental Pathology. The position will entail research and teaching to medical students and to graduate students. There are ample clinical opportunities for research programs needing them. The major areas of interest in the Division are immunology and tumor biology.

The level of academic appointment will be commensurate with the candidate's qualifications.

Reply with Curriculum vitae to: Thomas J. Gill, MD, University of Pittsburgh School of Medicine, Department of Pathology, Room 716, Scaife Hall, Pittsburgh, PA 15261.

An equal opportunity/affirmative action employer. W495(A)

UNIVERSITY OF PENNSYLVANIA
POSTDOCTORAL FELLOW

to study Control of Gene Expression and Steroid Hormone/Chromatin Interactions in Cultured Human and Murine Mammary Tumour Cells

Applicant must have strong bio-chemistry background. Experience with fractionation techniques, nucleic acid hybridization, steroid hormones, antibody production, and/or tissue culture desirable. Salary: $13,000.

Send résumé, three letters of recommendation and university record to Dr Fred R Frankel, 249 Johnson Pavilion G2, University of Pennsylvania, Phila. Pa. USA 19104.

W570(A)

Soil Scientist/ Metabolism Chemist

Jealott's Hill is the centre for pesticide research and development work carried out by the Plant Protection Division of ICI.

There is a vacancy with our Environmental Sciences Group for an enthusiastic person to lead a team studying the physicochemical behaviour and degradation of pesticides in soil and aquatic sediments. The person must have qualifications at the PhD level (or equivalent), a good knowledge of soil science and experience in studying the behaviour of chemicals in soil. An agreeable personality and an ability to work as a member of a team are also essential. The work is part of our programme for evaluating the environmental safety of pesticides.

Initial remuneration in the region of £8,500 per annum could be offered to the right person, and the Company has a Profit Sharing Scheme and a contributory Pension Scheme. Assistance with removal expenses and house purchase may also be available.

If you wish to apply, please write or telephone for an application form to:

Mr D Spence, Personnel Officer, ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire.

Telephone Bracknell 24701 extension 3445. Please quote reference number 79/SS/MC 3823(A)

ICI Plant Protection Division

NATURE Book Review Editor

Nature reviews a wide range of scientific books every week and several times a year also produces a major review supplement. The Book Review Editor is responsible for the selection of books, the commissioning of reviews and (in collaboration with typesetter) the preparation of all review pages for the printer. The Book Review Editor is part of the editorial team and will often call on other members of this team for advise; equally he/she will frequently be called on to advise and help these other members.

Applications (including a curriculum vitae) should be sent as soon as possible to — The Editor, Nature, Macmillan Journals Limited, 4 Little Essex Street, London WC2R 3LF.

3857(A)

DIABETES RESEARCH INSTITUTE

Biochemist or Biologist

Applications are invited for a postdoctoral research position. The research concerns electron microscopic studies on biological membranes and macromolecules. The applicant will work within the electron microscopic group.

Applications, including a curriculum vitae and names and addresses of 2 referees should be sent to *Gesellschaft zur Förderung der Erforschung der Zuckerkrankheit,* 4000 Düsseldorf 1, Auf'm Hennekamp 65. W568(A)

Australia

The University of New England

Lecturer

Department of Ecosystem Management

The Department provides Undergraduate and Postgraduate training within the Faculty of Resource Management, in the investigation and management of Biological resources. A new appointment is to be made, to contribute mainly to teaching and research in Wildlife Biology, Technology and Management. The successful applicant may be required to teach in related fields also.

Applicants should have a training in quantitative terrestrial, vertebrate ecology, preferably with a working knowledge of plant ecology, professional experience of wildlife management or of applied wildlife research, evidence of active field research and a PhD teaching experience and a knowledge of Australian fauna would be advantageous.

Salary: $16,291 — $21,401 per annum.
Closing date: 14th January, 1980
Position No.: 455

Applications, including the names and addresses of three referees and stating the position No. should be sent to the **Staff Officer, the University of New England, Armidale, New South Wales, 2351,** prior to the closing date.

3828(A)

nature

London and New York

3 Dyers Buildings, London EC1N 2NR, 01-831-6901

Suite 832 50 Rockefeller Plaza, New York NY 10020, (212) 765-5758

COMMONWEALTH AGRICULTURAL BUREAUX

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the Commonwealth Bureau of Horticulture and Plantation Crops, East Malling Research Station, Nr. Maidstone, Kent

Duties: Preparing abstracts in English for the journal *Horticultural Abstracts*, which covers world scientific literature in this field, indexing and answering scientific enquiries.

Qualifications: A degree in horticulture, agriculture, botany, or related subject, plus a reading knowledge of at least one European language (German preferred) and the ability to write good English. Applicants must be able to render the content of scientific publications into concise, accurate and readable abstracts. It is expected that the successful candidate will wish to make a career in the field of scientific literature rather than research.

Salary: In the scale £3289 to £6211, plus a compensatory allowance (taxable but not superannuable) of 3.3 per cent of basic salary to offset personal contribution to superannuation scheme. Starting salary according to qualifications and experience. Promotion to higher scales on merit.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: 23 December 1979. 3818(A)

UNIVERSITY OF NATAL

DEPARTMENT OF BIOLOGICAL SCIENCES

Durban

Applications are invited from suitably qualified persons, regardless of sex, religion, race, colour or national origin for appointment to the post of:

PROFESSOR AND HEAD OF DEPARTMENT

No particular preference exists regarding the research orientation of the successful candidate. It may be botanical, zoological or specialised within either of these disciplines. Equal consideration will be given to candidates with research interests in the cellular, physiological or environmental disciplines, but the candidate must show a strong interest in the presentation of integrated biology.

Salary in the range of: R15,000 to R18,000 per annum.

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, 4001, South Africa, with whom applications, on the prescribed form, must be lodged not later than 14th January 1980, quoting reference Adv. D94/79. W564(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF ENGINEERING SCIENCE

SENIOR EXPERIMENTAL OFFICER

A vacancy exists, from 1 January 1980, for a research and development engineer to work for a period of two years on the development of an instrument to measure accurately the movements of normal and abnormal human-knee joints. The engineer will work in the Engineering Science Department in Durham, but will have the support of the Departments of Orthopaedics and Medical Physics in Newcastle. The project has the financial support of the DHSS.

Applicants should be graduates in engineering or another appropriate subject, preferably with experience of instrumentation.

For a successful applicant with two years postgraduate experience the initial salary will be in the range £4,333 to £4,910.

Applications (3 copies) naming three referees should be sent by 31 December 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham, from whom further particulars may be obtained. 3844(A)

STUDENTSHIPS

ST. JOHN'S COLLEGE CAMBRIDGE

Research Studentships

The College invites applications for a number of Research Studentships normally tenable for three years and of such value as will, in addition to the payment of fees, bring the student's total emoluments to £1,920 a year, for men who are not already members of the College but who propose to register as PhD students at the University. Candidates should have gained or have a strong prospect of gaining a first-class honours degree or equivalent.

Further details and application forms can be obtained from the Senior Tutor, St. John's College, Cambridge CB2 1TP. Completed applications must be received by April 1, 1980. 3856(F)

FELLOWSHIPS

SIDNEY SUSSEX COLLEGE, CAMBRIDGE

LINNETT-BP JUNIOR RESEARCH FELLOWSHIP IN PHYSICAL CHEMISTRY

The College Council hopes to make an election to a Junior Research Fellowship tenable from 1 October 1980 for three years. The Fellowship will be funded by British Petroleum.

Candidature will be restricted to those pursuing, or intending to pursue, research in the broad area of the chemistry and physics of solids and their surfaces.

Applicants who will be over thirty years of age on 1 October 1980 are unlikely to be elected except in special circumstances.

The pensionable emolument of a Junior Research Fellow is currently £3,555 a year, or £3,964 if the Fellow holds the degree of PhD, subject in either case to a reduction of £570 if the Fellow is normally resident in College and also to such reduction as the Council may determine on account of any emoluments received from other sources.

Further information may be obtained from the Senior Tutor, Sidney Sussex College, Cambridge, CB2 3HU.

Applications should reach the Senior Tutor not later than 18 January, 1980. 3850(E)

EMBO

European Molecular Biology Organisation

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

SPRING 1980 AWARDS

Next deadline: February 18, 1980

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but, subject to review of progress by the selection committee, they are usually renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is February 18th 1980. Successful candidates will be notified of their awards immediately after the meeting of the selection committee which is on April 18th 1980.

Application forms and further details may be obtained from Dr J Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany. W563(E)

UNIVERSITY OF OXFORD

The Queen's College

JUNIOR RESEARCH FELLOWSHIP IN CHEMISTRY

The Governing Body proposes to elect to a Junior Research Fellowship in Chemistry tenable for three years from 1 October 1980.

Further particulars may be obtained from the College Secretary, The Queen's College, Oxford. 3819(E)

FRIEDRICH MIESCHER-INSTITUT, BASEL

POSTDOCTORAL FELLOWSHIP

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Send C.V. and list of publications to Dr R R Bürk, Friedrich Miescher-Institut, P O Box 273, CH-4002 Basel, Switzerland. W578(E)

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DEPARTMENT OF IMMUNOLOGY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP

Candidate should have experience in biochemistry, cell biology or immunology and a research interest in the cellular basis of immune reactivity.

The position is available for 2 years at a salary ranging DM38,000 to 40,000 per annum.

Applications with c.v. and names of referees should be sent to Prof. Eckehart Kölsch, Dept. of Immunology, University of Münster, Westring 10, 4400 Münster, Germany. W567(E)

POSTDOCTORAL FELLOWSHIPS IN ASTRONOMY AND ASTROPHYSICS AT CALTECH

The California Institute of Technology invites applications for postdoctoral fellowships in astronomy and astrophysics. Awards, beginning generally in summer or autumn 1980, will normally be for one year with likely renewal for a second year. Caltech is active in observational astronomy (radio, millimeter, infrared, optical, X-ray, and cosmic ray; solar, planetary, interplanetary, stellar, interstellar, and extragalactic), laboratory astrophysics (nuclear, atomic, and chemical), and theoretical astrophysics (with emphasis on cosmology, galaxies, stars, coherent emission processes, and nuclear and relativistic astrophysics).

It is anticipated that postdoctoral fellowships (number in brackets) will be available in the following fields:

1) Solar Physics (1): H Zirin, 354-33.
2) Experimental and Theoretical Cosmic-Ray Astrophysics and Solar-Terrestrial Physics (3): E C Stone, Downs Laboratory, 220-47.
3) VLBI Radio Astronomy (1): M H Cohen, 102-24.
4) Experimental (1) and Theoretical (1) Nuclear Astrophysics: W A Fowler, Kellogg Radiation Laboratory, 106-38.
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7) Quasar Astronomy (1): M Schmidt, 105-24.

Caltech is an Equal Opportunity Employer with an Affirmative Action Program. We encourage applications from women, members of minority groups, and persons with non-disqualifying handicaps.

Applicants should write for applications forms to the appropriate faculty member listed above at: California Institute of Technology, Pasadena, California 91125. All application material should reach the California Institute before January 15, 1980. W551(E)

ASSISTANTSHIPS

THE UNIVERSITY OF LANCASTER
DEPARTMENT OF ENVIRONMENTAL SCIENCES

Applications are invited for a Research Assistantship/Associateship in the Lunar and Planetary Unit starting on 1st January 1980 and lasting for a period of one year. The person appointed would automatically become a Co-Investigator in the Heat Capacity Mapping Mission of NASA and would work with the Principal Investigator at Lancaster on Satellite Monitoring of Sea Surface Pollution in the seas around the UK. The work would be mainly laboratory centred, involving the use of existing image processing equipment and a PET microcomputer. The principal aim of the research is to report on the feasibility of detecting oil spills in the North Sea. This aim will be achieved through the examination of new satellite images taken in the infra-red.

The appointment will be made in the salary range £3,775 to £6,355 (basic), according to qualifications and experience.

Applications (six copies) naming 3 referees, should be sent to the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, by 7th December, 1979. Further information about the duties can be obtained from Dr G Fielder, Department of Environmental Sciences (Engineering Building).

3841(P)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BOTANY

Applications are invited for a NERC Post-Doctoral Senior Research Assistantship to investigate reproductive strategies in *Fucus* species. Candidates should have research experience in intertidal ecology and/or botany. Initial salary £4,333 per annum.

Applications, together with the names of three referees, should be received not later than 14th December 1979 by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref RV/850/N.

3813(P)

CITY OF LONDON POLYTECHNIC
DEPARTMENT OF BIOLOGICAL SCIENCES
RESEARCH ASSISTANTSHIP IN NEUROBIOLOGY (ILEA)

Applications are invited from well qualified graduates in Biology or Physiology for the above position to commence as soon as possible. The research project involves the histological and physiological analysis of the neural mechanisms underlying acoustic communication in insects. Much of the work will involve micro-electrode recording from primary and central neurons.

The appointment will be tenable for two years in the first instance and will be extended to three years on satisfactory progress. The successful candidate will be expected to register for a higher degree with the CNAA under the supervision of Dr D B Lewis.

Salary: £3,321 to £3,417 to £3,513 (includes London Weighting of £474 pa).

Please apply in writing, giving full curriculum vitae and the names and addresses of two referees, to Dr D B Lewis, Department of Biological Sciences, City of London Polytechnic, Old Castle Street, London E1 7NT. Applications should be sent within two weeks of the appearance of this advertisement. 3815(P)

CONFERENCES

STREAM ECOLOGY SYMPOSIUM
October 19-22, 1980
Augusta, Georgia USA

The Savannah River Ecology Laboratory, in cooperation with the University of Georgia's Institute of Ecology, the US Dept of Energy, and the National Environmental Research Park program, will host a symposium on the ecology of lotic ecosystems. Symposium format will include presentations by invited speakers, panel discussions, and contributed papers. Abstracts due June 1; manuscripts due August 1, 1980.

For registration and additional information contact: Thomas D. Fontaine, III, PhD, or Steven M. Bartell, PhD, Co-chairpersons, Savannah River Ecology Laboratory, PO Drawer E, Aiken, South Carolina 29801, USA. Phone: (803) 725 2472. W574(C)

SEMINARS and SYMPOSIA

Scottish Electrophysiological Society
3rd International Symposium
to be held at Edinburgh University 8-11 April 1980

Making sense of sense organs

The Symposium will present expert opinion on sensory receptors and how they work. The plenary sessions presented by invited speakers from the UK, USA, Germany, France and Holland will develop a theme from anatomy at ultrastructural and cellular levels, through the biophysics of stimulus absorption, transduction and coding to higher-order analysis and perceptual mechanisms of behaviour.

Voluntary contributions are cordially invited for the afternoon sessions and a poster session.

Please address enquiries to Dr Derek Cosens, Dept. of Zoology, West Mains Rd., Edinburgh EH9 3JT; or Professor Mike Laverack, Gatty Marine Lab., St. Andrews, Fife KY16 8LB, Scotland.

3825(M)

COURSES

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY
MSc IN TOXICOLOGY

Applications are invited for a postgraduate course leading to an MSc degree in toxicology. The course, which may be attended either fulltime (extending over one year starting in October) or part-time (extending over 2 years) is organised by the Department of Biochemistry, University of Surrey, with the MRC Toxicology Unit, Carshalton; the British Industrial Biological Research Association, Carshalton and Shell Toxicology (Tunstall) Laboratory, Sittingbourne. During the course instruction is given in these collaborative laboratories as well as at other major toxicological institutions. A limited number of MRC studentships are available and for suitable students these may be extended for continuation to study for a PhD.

The Medical Research Council, the Royal Society and the Royal College of Pathology have identified toxicology as a priority area for research training and therefore support the encouragement of first class students from a variety of disciplines to become involved in the science of toxicology. In modern society contact with potentially toxic chemicals is increasing. Problems may arise from the use of drugs for medical purposes, from occupational exposure to industrial products or intermedites and from environmental exposure to natural or synthetic substances.

The course, which was the first of its kind in Europe, is designed to provide graduates in science, and medical and veterinary graduates, with an understanding and appreciation of the many disciplines involved in toxicology. Particular emphasis is placed on aspects related to the biochemical mechanisms of chemically-induced toxicity, and the assessment of toxicological hazard in the manufacture, use and disposal of new chemical compounds, pesticides and medicines. The course provides a broad biological education and is a suitable introduction for those intending to undertake research on the interaction of chemicals with biological systems.

There are numerous interesting opportunities for scientists, educated initially in different disciplines, who have acquired additional knowledge of toxicology. These may be in industry, government regulatory bodies, or research institutes with good career prospects in both laboratory work and administration.

Further information and application forms to be returned by February 16th can be obtained from:
Dr R S Jones, Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH. 3851(D)

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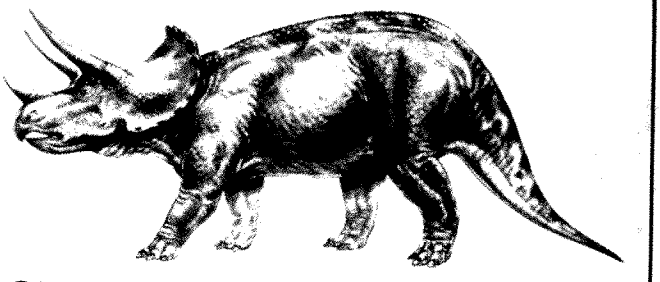
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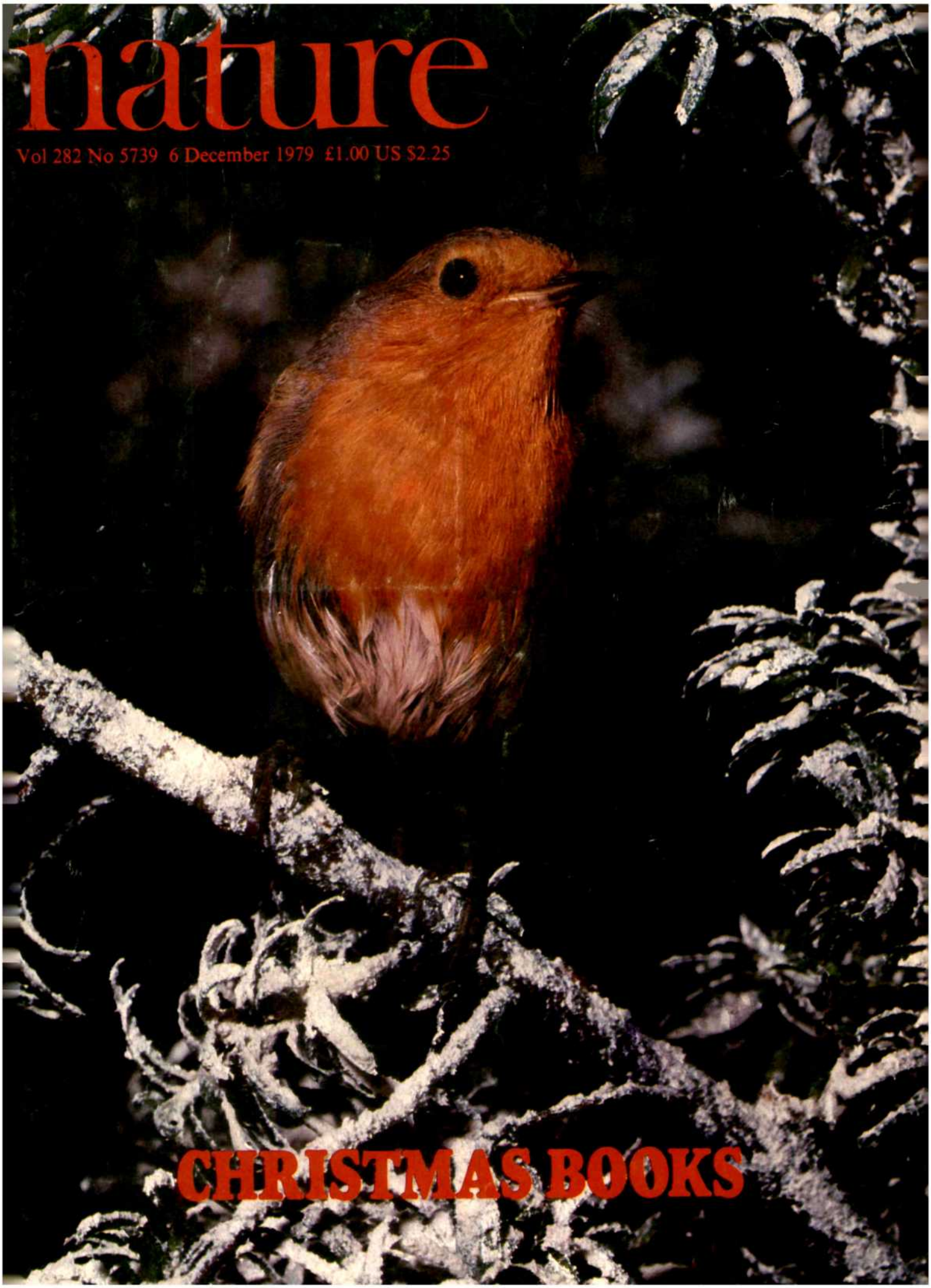
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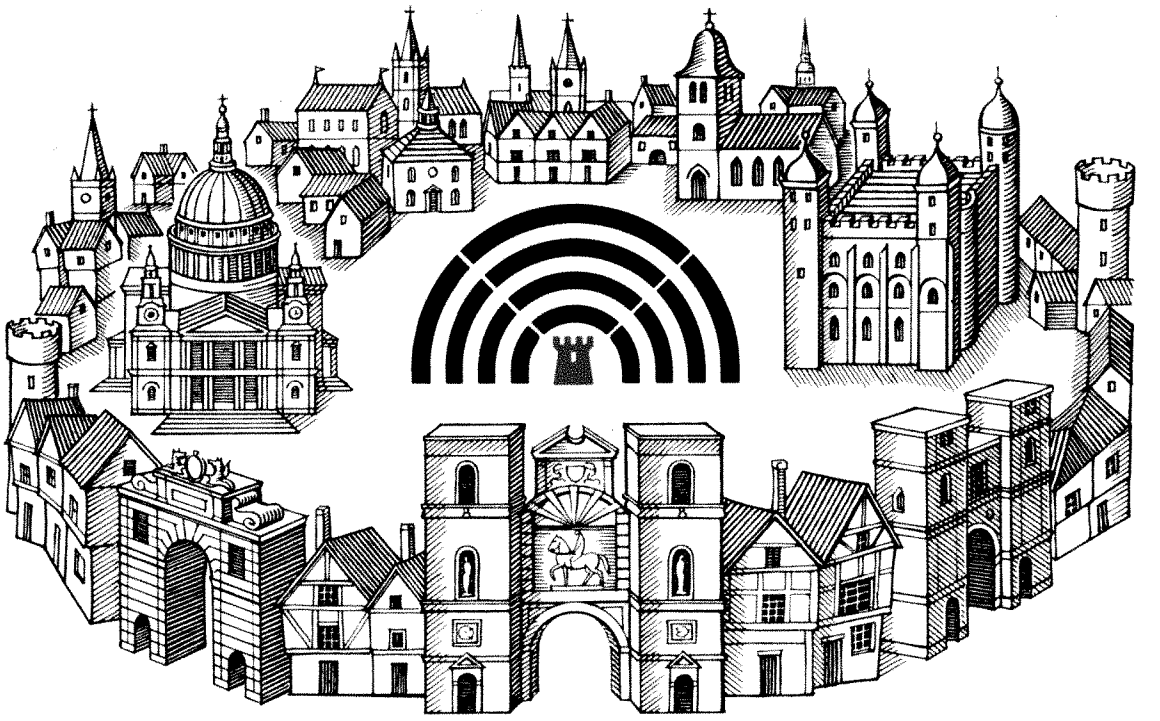
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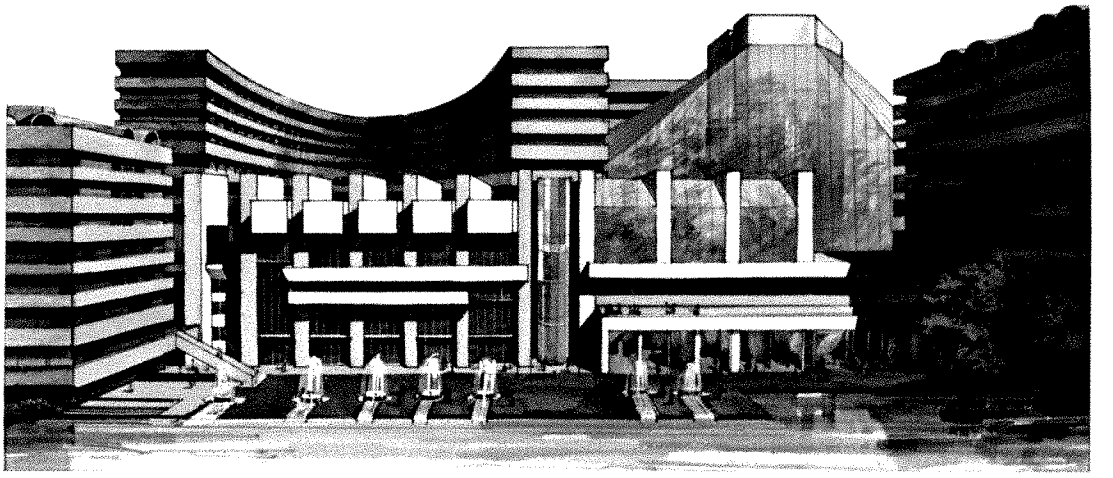
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Cover picture

This year's review of Christmas books (starting on page 631) includes three sets of books centred on birds — conservation, bird paintings and photographs, and birdwatching guides
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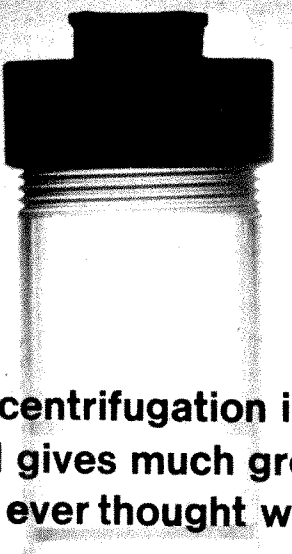
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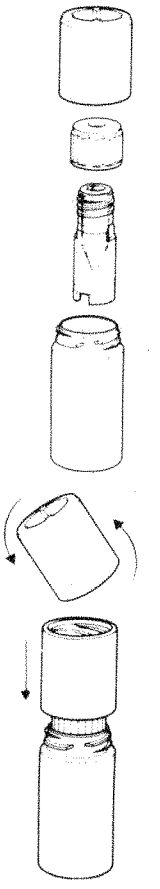
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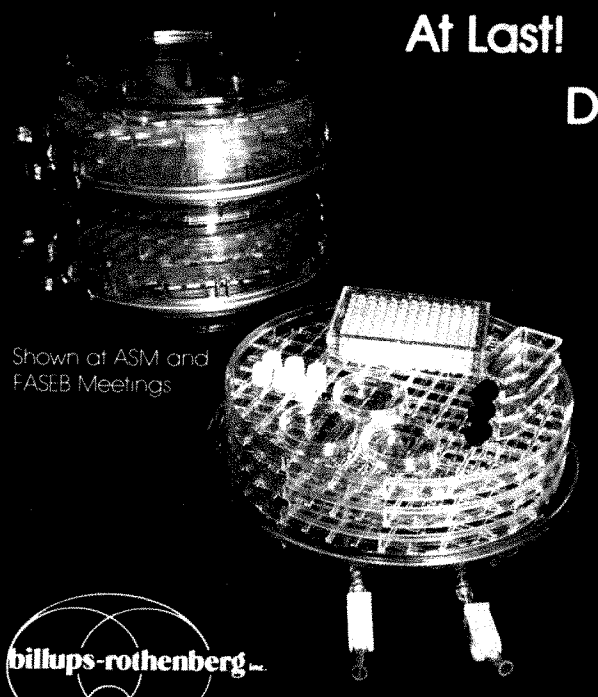
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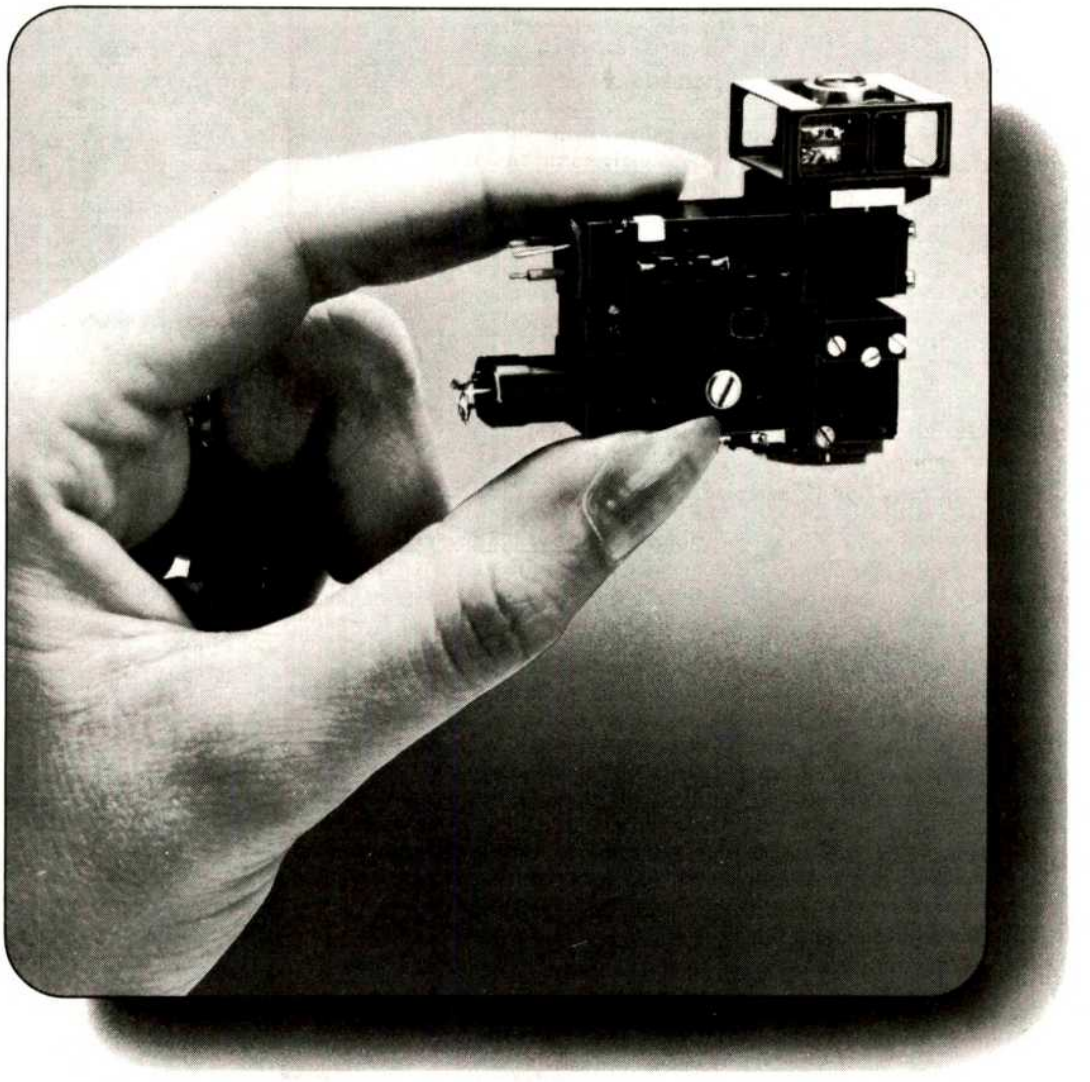
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Cutting laser technology down to size

The world's first diode laser optical recording system, developed by Philips, is remarkable, not only for what it can do, but also for how it does it.

For in order to record and/or retrieve 10^{10} bits of information on a pre-grooved, double-sided, 12-inch disk, a breakthrough in state-of-the-art optical recording technology was required. Which is precisely what we achieved.

The optical read/write head shown here is a miracle of micro-precision engineering. Employing an AlGaAs (Aluminium Gallium Arsenide) diode laser and associated semiconductor modulator, it produces sufficient pulsed light output to write, or 'burn', 1-μm data pits into the disk's tellurium-based recording film. A 40-gram optical system, as well as electro-optics for tracking and focusing are also



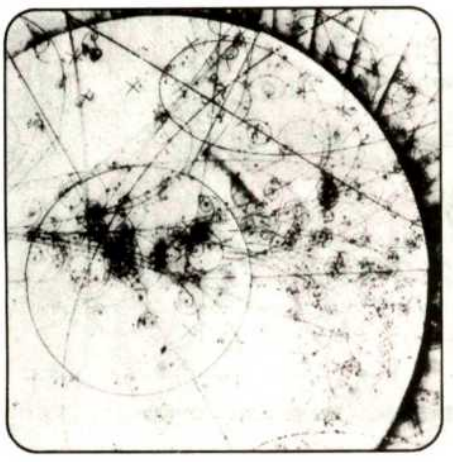
PHILIPS

contained within the ultra-compact head.

Light output from the laser is split by the optical system into two beams: 90 percent intensity for recording and 10 percent for reading. The read beam passes two mirrors and a beam splitter that are arranged to recombine the record and read beams at the objective, which focuses onto the information layer in the disk with an accuracy of 1-μm.

And to complement the precision and compactness of the optical read/write head, equally ingenious radial tracking and error-detection correction systems had to be developed.

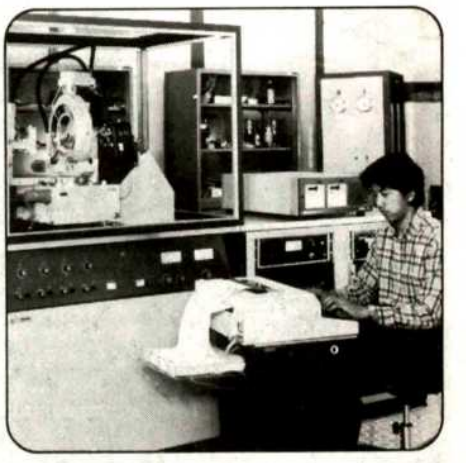
Diode laser optical recording is one example of Philips' ability to translate advanced technology into practical innovation. Here are some others.



CERN, the Geneva-based European Organisation for Nuclear Research, is to install four 500kW, 200MHz power amplifiers for their Super Proton Synchrotron, which is currently operating at a level of 400GeV. The new power amplifiers will provide additional RF power to double the Synchrotron's present capacity to facilitate experiments in high energy physics. Each of the four amplifiers employs 17 air-cooled coaxial power tetrode tubes of metal ceramic construction; power output per tube being approximately 37.5kW.
The contract, awarded to Philips, calls for the supply and installation of the amplifiers as well as supply of spare tubes for a period of ten years.



Fibre optics transmission. Trials on a long haul fibre optics telephone transmission route, covering a distance of ninety-six kilometres, has been successfully completed at the Philips laboratories in the Netherlands. Essentially, the route comprises a 19mm diameter cable containing six 0.1mm fibres with a regeneration stage every eight kilometres.



Tokyo Institute of Technology has installed a Philips PW1100 computer-controlled Single-Crystal Diffractometer to assist in structure analysis of inorganic materials. One of Japan's leading universities, the Tokyo Institute of Technology, is noted for its academic research into the field of inorganic materials. And the new installation will be used by crystallographers from all over the country.

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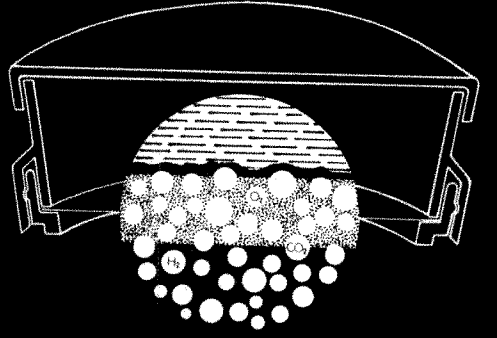
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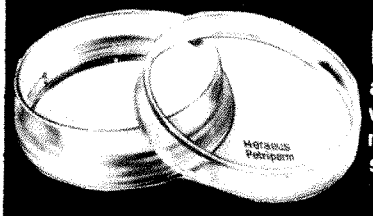
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Philips working on advanced technology

A new approach in tissue culture





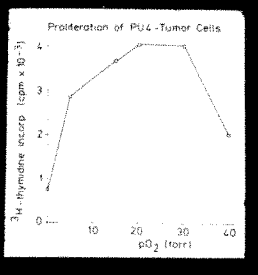
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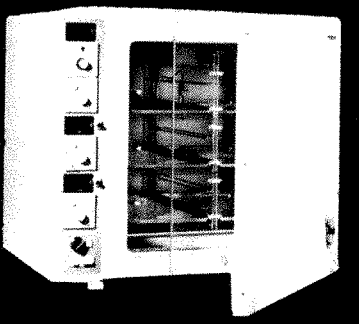
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6 December 1979

Public interest representation on research councils?

Who runs Britain's science? A simple question capable of an exceedingly complex answer, so widely is science dispersed across the scene. But for university scientists and many others in specialist laboratories there is a relatively simple answer: the research councils, which fund individual research projects, provide studentships and fellowships and operate a large number of major laboratories, many with an international reputation.

And who sits on the research councils and on the Advisory Board for the Research Councils, which coordinates their efforts and provides advice to the Secretary of State for Education and Science on how to spend £300 million annually? Distinguished scientists and medical men, a handful of industrial scientists and one or two farmers (on the Agricultural Research Council). Council members are appointed by the Secretary of State.

Now to the best of our knowledge members of the research councils fulfil their duties diligently, giving up time that they can often ill afford to attend meetings, understand complex issues and help to steer policy. We do not question the effort that they put in. What should be questioned is whether the composition of the councils is right for the 1980s, and whether there should not be a greater representation of what, for want of better words, may be called the public interest.

Before we are accused of mischievously allowing amateurs and meddlers to interfere with such delicate issues as who gets grants, it should be made clear that each research council has a complex structure of committees which handles such matters; council only concerns itself with broader issues. But these could profit by being understood and debated by people with an interest in science but who have not necessarily followed the research path.

There is no shortage of constituencies which could make valuable contributions. They could include trade-unionists, teachers, those in overseas development, politicians, science writers, lawyers, businessmen, bankers and doubtless many others. Since much of science is international, there should be no compelling reason why a foreigner (particularly from the EEC) could not be incorported for some purposes — one or two other countries already do use foreign help in deciding science policy.

Of course scientists themselves would have to be protected against being swamped by non-scientists, but the model of the Genetic Manipulation Advisory Group, which satisfactorily incorporates several 'public interest' members, would bear study. One of the weaknesses of Britain is the lack of cross-communication between experts in different disciplines. Here is a chance for an experiment in trying to build up such cross links. □

Flying university in trouble

HIGHER education and scholarship in Poland recently acquired an unusual and innovative institution — the Society for Academic Courses, better known by its nickname of the 'Flying University'. This society arranges lectures, seminars and discussions to satisfy the needs particularly of young students given to questioning the information obtainable through conventional channels. Although a large fraction of the courses currently on offer concerns contemporary history and the social sciences, scientists are amongst the sponsors of the society, and a course is included on moral issues in biology. The society has received notable public support from physicist Janusz Groszkowski, former chairman of the Polish Academy of Sciences, who resigned his official posts in 1976 in protest against police interference in academic life.

The educational experiment has not gone entirely smoothly. The police had been regular visitors to houses where seminars are held, and several lecturers have been detained and fined on various charges of disturbing the peace. Seminars have also been visited frequently by gangs of students intent on doing damage. This term only the inaugural session, by the sociologist Witold Bartoszewski and the physiologist Jan Kielanowski (a member of the official Polish Academy of Sciences) was given before the wreckers moved in. Two of those involved, Dr Bartuszewski, and Piotr Naimski, a biochemist, have each been fined 5000 zloty — the equivalent of an average month's academic salary — for their activities.

An international support committee has just been formed to provide encouragement for an independent intellectual life in Poland by the provision of material help, including books, and by the publication of research results. It is a cause that scientists who have a large amount of freedom to say and do what they wish may wish to contribute to.

The address is 11 Bevington Road, Oxford. □

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NASA told to delay space mission with European scientists

A US congressional committee last week directed the National Aeronautics and Space Administration to impose a two-year delay on a joint solar research project with European scientists, the International Solar Polar Mission.

The House Appropriations Subcommittee responsible for NASA's budget said the delay was necessary because of difficulties faced in the development of the space shuttle, from which two spacecraft would be launched simultaneously to orbit the sun in opposite directions.

In addition, the subcommittee has told NASA that both the solar polar mission and the planned Project Galileo mission to Jupiter, which the agency has already suggested postponing from 1982 to 1984, should use modified Centaur rockets for the launch from the shuttle, rather than the 'inertial upper stage' (IUS) currently under development by the US Air Force.

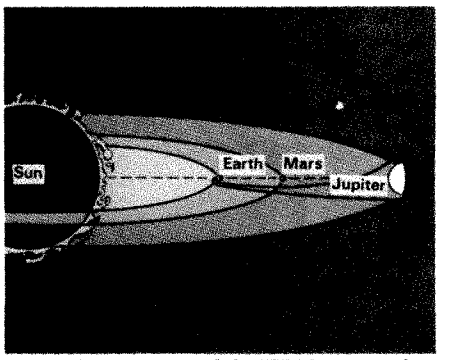
The instructions were contained in a letter to Dr Robert Frosch, administrator of NASA, from Appropriations Subcommittee chairman Mr Edward Boland. Two years ago Mr Boland tried to persuade the House of Representatives that Project Galileo should be killed entirely, and was only defeated after intensive lobbying by the space science community.

The legal status of the directive is unclear, since any change of plan would need to be endorsed by at least three other congressional committees. But with the space shuttle running into increasing delays and rising costs, space science projects have become vulnerable prey to budget-cutting politicians.

The solar-polar mission is a joint effort between NASA and the European Space Agency. The two spacecraft are currently scheduled to be launched in February 1983, and after a fly past Jupiter to enter polar orbits around the Sun in 1987.

Problems have arisen because delays in the shuttle development programme — now over a year behind schedule — have contributed to difficulties in guaranteeing that the IUS could be developed sufficiently fast to provide the thrust needed to launch the two spacecraft from the shuttle by the planned date.

Similar problems have affected the Galileo project, which is also planned by NASA to use the IUS, and for which NASA has now decided that there should be separate launches for the probe and the orbiter in 1984. Previously it had been planned that both vehicles would be launched simultaneously by the IUS, but this could only have been done with a 1982 launch making use of the extra pull from the planet Mars.



Planned trajectories of the ISPM spacecraft: a 2-year delay will add greater strain to ESA's badly stretched space science budget.

Last summer NASA had rejected the Centaur as an alternative to the IUS, largely because of the extra development costs, which it calculated would have added an extra $100 million to the cost of Galileo, whose predicted budget has already risen from $450 million to $675 million because of the time delay.

In his letter to Mr Frosch, however, Mr Boland says that his subcommittee will only let Galileo proceed "if NASA develops a Centaur upper stage designed to fly a combined orbiter-probe Galileo mission in 1984", and that the solar-polar mission, also using the Centaur, must be put back to 1985.

ESA officials point out that, under their agreement with NASA, the US agency would have to bear any additional launch costs for the solar-polar mission. However they are seriously worried about the effects of a two-year delay, not only because of the extra costs of keeping scientific and technical teams together, but because it could initially mean the underutilisation of a small space science budget, as well as upsetting medium-term budget priorities.

NASA received more bad news last week, when the Office of Management and Budget rejected several proposals for new programme starts which the agency had submitted for possible inclusion in the President's budget request to Congress for the fiscal year 1981. The only new start to have received OMB's blessing is said to be the proposed launching of a gamma ray observatory. However the budget office has not supported NASA's request to start development work on a propulsion system necessary if a joint mission is to be carried out for encounters with Halley's Comet and the Comet Tempel 2 in 1985.

NASA officials are now hoping that the White House can be persauded to support some of the projects deleted by OMB. In particular discussions are taking place over joint sponsorship between the Department of Defense, the Commerce Department, and NASA about establishing a national oceanic satellite system, a successor to the ill-fated SEASAT. □

UN split over science policy machinery

Member countries of the United Nations remain sharply divided over the type of internal machinery that the UN should set up to help implement decisions reached by the Conference on Science and Technology for Development (UNCSTD) held in Vienna in August.

At stake is the future of the Office of Science and Technology, currently the prime focus for science policy activities within the UN, but which many developing countries consider inappropriate, in its present form, to an expanded role.

Delegates attending the second committee of the UN General Assembly in New York last week, which is examining the conclusions of the conference, voiced unanimous approval for its decision to establish a new Intergovernmental Committee for Science and Technology for Development (IGC) open to all member states of the UN.

As in Vienna, however, opinion is divided over the nature of the secretariat which will service this committee. Many developed countries, and in particular the nine member countries of the European Economic Community, expressed their opposition to any new institutional machinery. They argue that the IGC could be adequately serviced by a strengthened department of International Economic and Social Affairs, under which OST now operates.

The Group of 77 however, which has been negotiating for the developing countries, has called for the abolition of the OST, and for its function and resources to be transferred to a new, independent secretariat, headed by an under-secretary general directly responsible to the Director-General for Development and International Economic Cooperation, Mr Kenneth Dadzie.

Mr Dadzie has himself offered a compromise solution which would create a new secretariat, but keep some resources within the Department of International Economic and Social Affairs. However many delegates feel that a clear division of responsibilities is necessary, and Mr Frank Da Costa, Secretary-General for the UNCSTD conference, has argued strongly that the Group of 77's proposal should be accepted.

After two days of open session, the second committee adjourned for informal discussions on the alternative proposals. A final decision is expected next week.

In addition to servicing the IGC, the new secretariat would be responsible both for co-ordinating science and technology activities within the UN system, and for providing support to the group of experts which the IGC will set up to study plans for a new financing system for science and technology for development. □

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Recombinant DNA experiments to remain under safety guidelines

There will be no major removal of experiments using recombinant DNA techniques from safety guidelines laid down by the National Institutes of Health, writes **David Dickson**

Last week the director of NIH, Dr Donald Fredrickson, proposed in response to a recommendation from the Institute's Recombinant DNA Advisory Committee (RAC) that physical containment levels for virtually all experiments using the disabled K-12 strain of the bacterium *Escherichia Coli* should be reduced to the P1 level.

However he rejected the committee's suggestion that the experiments be formally exempted from the guidelines — and hence from NIH enforcement — proposing instead that they be placed in a special class which would still ensure that the containment requirements were met.

Dr Fredrickson's proposals were announced in the *Federal Register* last Friday (30 November) and follow a close scrutiny of the reasoning which led to the RAC's decision, taken by a vote of ten to four, with one abstention, at the committee's September meeting.

Rather than giving precise reasons for his decision, Dr Fredrickson lists the various events necessary for a serious accident to occur. In each case he quotes the opinion of scientists that the chance of such an occurrence is relatively low; and he points out that when a series of small probabilities are multipled together, the chance of a sequence of events is "exceedingly low".

On the question of whether *E coli* could cause an epidemic by passing from one person to another, for example, he quotes the conclusion of Dr Eugene Gangarosa, writing in a recent issue of the *Journal of Infectious Disease* that an epidemic "does not seem remotely possible".

Similarly in response to the question of whether *E coli* K-12 is pathogenic, Dr Fredrickson quotes Dr Sherwood Gorbach's conclusion that the disabled bacterium is intrinsically impaired in most, if not all, of a variety of virulence factors, all of which are required by a micro-organism to produce disease.

Taken together, suggests Dr Fredrickson, this evidence is sufficient to reduce containment levels for the bacterium to P1. However he says that he is not proposing to exempt the experiments, since the term 'exempt experiment' as currently used in the guidelines "should only be used for experiments for which no containment level is specified and for which no registration with the IBC is required".

As an alternative, Dr Fredrickson has proposed creating a special class within the guidelines for experiments using *E coli* K-12, except for otherwise exempt or prohibited experiments, or as otherwise specified. Experiments in this class would require P1 containment including a ban on mouth pipetting and the requirement that all biological wastes are decontaminated.

"For these experiments no memorandum of understanding and agreement . . . need be submitted, nor is any registration with NIH necessary."However, suggests Dr Fredrickson, for these experiments, prior to their initiation, investigators must submit to their institutional biosafety committee a registration document that contains a description of:

- the source(s) of DNA,
- the nature of the inserted DNA sequences, and
- the hosts and vectors to be used.

The IBC will review all such proposals submitted by investigators, although such review will not be required prior to initiation of experiments. An exception, however, requiring both prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E coli* K-12 efficiently express any gene coding for a eukaryotic protein.

Elsewhere in this proposed revision to the guidelines, Dr Fredrickson repeats his proposal to introduce a new section covering in detail the way in which private companies can register their experiments with the NIH and submit them where necessary for approval to the RAC, but on a voluntary basis.

Dr Fredrickson says that a number of objections to this process have been received by NIH, claiming a voluntary system of regulation to be inadequate. However he also points out that the RAC voted by 11 to none, with four abstentions, in favour of these proposals, and says that he is therefore intending to include them in the revised guidelines. □

US legislators reject moratorium on nuclear power

In its first significant vote on nuclear power since the Three Mile Island accident last March, the US House of Representatives last week rejected by 254 votes to 135 a proposal that the Nuclear Regulatory Commission be required to impose a six-month moratorium on issuing construction permits for new nuclear power plants.

In a similar vote last July, the Senate had also rejected a moratorium proposed by Senator Edward Kennedy by 57 votes to 35. Since then, however, a national poll has shown 57% of those interviewed to favour such a move — and supporters of the moratorium were therefore disappointed not to have done better in the House vote, where they had hoped to pick up considerably more supporters.

The moratorium proposals have been the major focus of public debate since the TMI accident, much of it symbolic since the NRC has itself announced that it will not grant any new permits until it has absorbed the conclusions of the President's Commission of Inquiry, chaired by Dr John Kemeny.

In presenting the Commission's report last month, Dr Kemeny said that eight out of the 12 members of the Commission had favoured a moratorium of some kind; but no more than six — less than the needed majority — could agree on specific conditions under which a moratorium would be terminated.

Last week's proposal was put to the House by Representative Edward Markey, a Democrat from a working-class district of Boston, who argued that it was not meant to be a pro-nuclear or an anti-nuclear statement, but merely to signify that nuclear power should be made safe.

The TMI accident demonstrated that the regulatory framework had failed, and fundamental reforms were needed to make nuclear power safe. The amendment "would send a message loudly and clearly to the industry and the Nuclear Regulatory Commission that Congress will no longer tolerate any future corner-cutting in the area of nuclear safety", Mr Markey said.

Opponents of the moratorium, however, argued that it was unlikely to have any significant effect — and that recent events in Iran had underlined the need for nuclear power to reduce dependence on foreign oil.

"The passage of the Markey amendment would be used by certain sectors of the media to dramatically harm public confidence in nuclear energy all across the world in the same way that they use the TMI accident to sensationalise and exaggerate nuclear hazards", said Mr Mike McCormack, chairman of the House Science Committee's Energy Subcommittee.

After the amendment had been defeated, Mr Markey said he still felt it was a good beginning "considering the 25 years of lobbying for nuclear energy and the very intensive effort by the utilities and the reactor manufacturers during the past few weeks".

In contrast Mr Carl Walske, President of the Atomic Industry Forum, said: "We think the House sent a very good message to the Ayatollah in voting it down". □

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West Germany to propose big new accelerator

TODAY physicists at the Deutsches Elecktronen-Synchrotron (DESY) hope to hear that they may go ahead with a proposal for a new two-stage, DM 700 million electron and proton collider to be built under a park near DESY in Hamburg.

The accelerator would be called HERA for 'Hadron Electron Ring Accelerator', and would provide collisions between 35 GeV electrons and 400 GeV protons. This would create the deepest probe yet of the three quarks which bind together to form the proton, and would take physicists one step forward to discovering whether the quark itself has structure.

If the West German ministry for research and technology (BMFT) and Hamburg City Council grant DESY leave to propose, HERA may be before the minister by January. Bjorn Wiik, who was behind a similar (but rejected) proposal at the European nuclear centre, CERN, has already progressed a long way with the design of HERA.

Furthermore, the BMFT is believed likely to turn a sympathetic ear to the project: the ministry is said to have a surplus of some DM 300 million of research money, and the Minister, Dr Volker Hauff, has frequently said that he wants to secure the future for DESY.

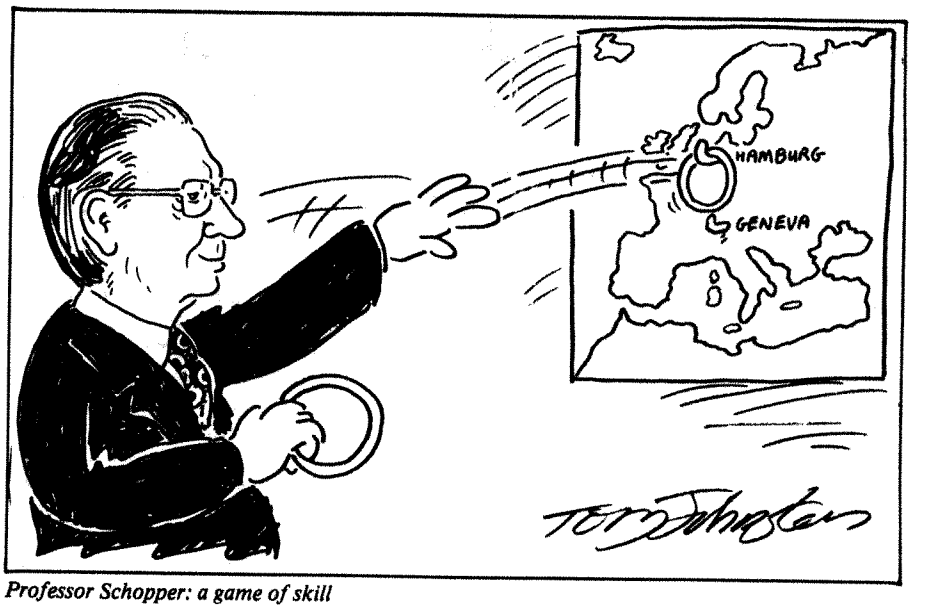
HERA is a project in two parts. The first is the building of a tunnel, deep in sand and the construction of an electron ring. "Surprisingly it is not easier to build a tunnel through sand than through rock" said a DESY spokesman. The tunnel would need concrete walls, magnifying the cost.

The cost of HERA, part one, would be DM 300-400 million. Part two involves the construction of a superconducting proton ring to accelerate protons to some 400 GeV; this would cost an equal amount.

It thus appears that the likely beginning of HERA will be a single electron ring of 35 GeV. This alone is of little use. But an electron ring one way round is a positron ring the other. So HERA will start by colliding electrons with positrons (their antiparticles) at a total energy of 70 GeV.

DESY insist that it would complement rather than compete with the large electron positron ring (LEP) that CERN hopes to build.

CERN's LEP would be designed with conventional magnets to reach an energy high enough to produce the Z^0, a particle predicted by the Weinberg-Salam unification of the weak and electromagnetic interactions. (The predicted mass of the Z^0 is around 90 GeV.) With superconducting magnets (and accelerator cavities, an unproven and complex technology) LEP would reach up to the 150 GeV or so needed to produce pairs of W^+ and W^- — the other prominent particles predicted by Weinberg-Salam.



Professor Schopper: a game of skill

HERA, on the other hand, without superconducting bending magnets or cavities, would not reach the Z^0. Its role would be to look at the 'interference' between the photon and the Z^0 in electrons — positron interactions. (When they interact through electromagnetism, electrons and positrons exchange photons; through the weak interaction, almost completely masked at low energy by electromagnetic effects, they exchange Z^0s). At HERA energies, the interference would be a maximum.

Superconducting magnets and cavities are not part of the plan for HERA's electron ring, says DESY; but if they do become available it would no doubt be tempting to DESY to raise HERA towards the Z^0. A team at Karlsruhe have already had some success in superconducting cavity design.

Nevertheless the true goal of HERA is e^--p collisons, not e^-e^+. "We don't want to take the Z^0 and the Ws from CERN" said the spokesman. "There is no war."

If HERA goes ahead, it would be likely to find international support, as have the present DESY electron rings DORIS and PETRA. The director of DESY, Professor Herwig Schopper, refused access to PETRA to groups composed purely of one nationality — including German. Thus researchers at DESY now include more non-German physicists than German. The French and British have the strongest representations at DESY; Japan, China and the US are also there. Italy, for the moment, is absent.

Schopper, however, may not remain director of DESY beyond the end of his term on 31 December 1980. On 1 January 1981, CERN itself needs a new Director-General, and Schopper is considered by many to be high on the list. CERN Council meets in two weeks — on 19 December — and the state of CERN-DESY relations at that moment may be assumed to be an important factor. DESY always raises the fear at CERN of competition for LEP, and the surreptitious announcement of HERA — within a closed meeting of the European Committee for Future Accelerators — has done little to dampen it. **Robert Walgate**

PETRA apprehensive of US rival

As PEP, the US rival to West Germany's PETRA electron-positron collider, approaches commissioning in the new year, PETRA physicists are concerned that PEP's likely higher event rate will eclipse the German machine.

PETRA has faced problems of resonances in the machine as it accelerates from its injection energy up to the experimental region. PEP will take injection at the experimental energy, and so avoids most of the resonances.

PETRA experiments have each collected "a few hundred" events in a year's operation. They have included evidence for the 'gluon' (the photon of the quark-quark force), and excited states of the upsilon (which contains a beauty quark and its antiparticle), but 'truth' (the quark beyond and pairing with beauty) remains to be found, as does Z^0-photon interference (which needs a 4-5 times higher event rate) and the details (*à la* charm) of the beauty spectrum.

In January, there are plans to boost PETRA to 36 GeV (total energy) by increasing the number of accelerating cavities from 32 to 60, but that will introduce yet more resonances. "If they get a factor five more events" said one DESY physicist this week, refering to PEP, "then we will be overtaken". This cloudy future may be one element encouraging DESY to make a strong play for HERA. □

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Universities should opt for teaching or research

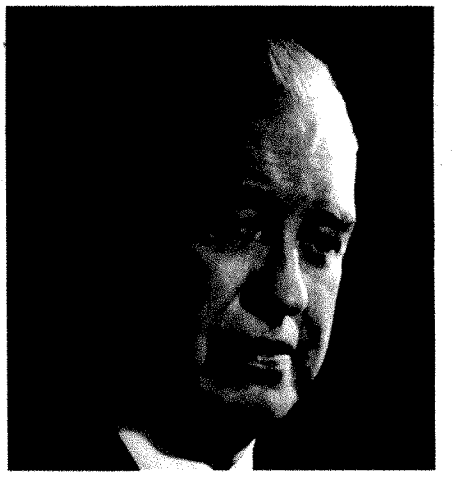
In his anniversary address to the Royal Society last week, Lord Todd, the Society's President, lamented the deteriorating health of Britain's science and argued for a restructuring of the university system. Here we publish extracts from his speech.

THE rapid growth of the university system in the 1960s brought about a vast expansion in tenured staff usually by the recruitment of relatively young men and women taken from the normal supply of university graduates and not invariably of the highest quality. Many, if not most, of these remain today at the same institutions and are likely to do so for perhaps another two decades under the tenure system.

A detailed study of chemistry departments in British universities has been made by Professor Colin Eaborn who summarised his findings thus: "At present only 7.8% of the staff of chemistry departments are below 35 years of age; the proportion will probably fall to about 4% in five years' time and rise to only about 9% in ten years time. The proportion below 40 years of age, now 26% will fall to about 12.5% in five years and to about 10% in ten years time. During those ten years the proportion of staff over 50 will rise from the present 28% to about 62%."

Viewing Europe as a whole, the European Science Foundation suggests that the chance for a junior research assistant to reach a permanent university appointment has gone down from about 70% during the 1960s to about 15% in this decade. The outlook is indeed bleak. Surprising though it may seem, the academic research profession has within a few years been transformed from one of the most mobile to one of the most static. This is particularly so in the United Kingdom, where the economic stagnation of recent years has all but staunched the flow of academics in mid-career into other occupations. Many young scientists are seeking to retain their present precarious positions by hand-to-mouth grants because they fear that a change to another university or research institute might jeopardize their chance of obtaining a permanent appointment.

Several awkward, even painful, questions arise. Is it, for example, to continue to be taken as an article of faith that all established academics are capable of first-class research and that all students obtaining first-or upper-second-class honours degrees should be encouraged and provided with the wherewithal to pursue academic research? The answer to both questions must, I fear, be no. If we continue to believe that each department in every one of our present universities should have a substantial research school the strain on our financial resources may become intolerable.



Lord Todd: revise the dual support system

The dual support system of the University Grants Committee and the Research Councils would ideally ensure that extra funds for the support of research were concentrated on those most likely to spend them well; as things now stand, however, while there is no reason to believe that really able young scientists with good projects to put forward will be denied temporary funding to initiate them, the prospects of their being given a sufficient measure of permanence to build up a centre of excellence in their institution is small.

It is not possible — and it may not even be desirable — that all departments of, say, chemistry or physiology should stand out for the high quality of their research. Some, for example, have too small a staff adequately to sustain undergraduate teaching and to supervise at the same time programmes of post-graduate work which all departments in all disciplines appear to regard as the essential breath of life. Need this requirement for large postgraduate programmes be universal?

It is a remarkable fact that in proportion to population there are more institutions awarding PhDs in physics in the UK than in the US. In the US we find many more institutions devoted to professional education and applied sciences and there are many distinguished universities whose reputation rests substantially on the quality of their basic teaching. Such diversity in our institutions should be encouraged. Why should there not be some differentiation between those who teach and those who pursue research?

Unfortunately the present machinery for the support of higher education and research in the United Kingdom was not devised for the encouragement of diversity. For all its undoubted virtues the University Grants Committee system does require that universities should compete within what is increasingly a common framework of objectives. So competition between them is almost always on familiar terms.

Preliminary (and anecdotal) evidence suggests that in this competition Oxbridge and the older civic universities are winning out. If this be the case and the trend continues then we will end up with a hierarchy in which the universities lower in the pecking order will be trying to do the same things as the others but doing them with students who are not suited to them and would do better and become more useful citizens with a more vocational type of education.

If we are to change our present university pattern, the mechanisms by which we support research in academic institutions may also have to undergo some changes. Project grant applications will no longer be assessed simply on their merits alone without reference to the circumstances under which the research is to be carried out. Research projects in a given field will tend to be concentrated in one, or in only a very few, centres with considerably larger research groups than are usual today. It may well become necessary for the research councils to be more selective in the way in which postgraduate studentships are allocated to university departments; more radically, they may even have to think of making the grants to students of exceptional promise rather than to their potential supervisors. Another convention that may have to go is that every reasonably able PhD graduate can expect as of right to have two or three years of postdoctoral research during which he can establish a claim on a tenured research post.

The frequent frustration of this expectation is one of the saddest of the current symptoms of malaise in our university research. But the vigour of our research enterprise would surely suffer if all those concerned were now provided with a formal tenured career structure as many of them are now asking despite the current staff structure in our universities.

Unfortunately there is no quick or easy answer. A reduction in the university retiring age to 60 without any reduction in pension would undoubtedly speed up return to a normal age distribution, but would be expensive. Encouragement of voluntary retirement at 55 with generous financial compensation has also been suggested but would probably not be welcomed by more than a few individuals, and would also be costly.

If we accept that some universities will be much more devoted to vocational teaching and less to research than others, then only in a proportion of our universities need the situation be treated as urgent. These urgent cases could have a retiring age of 60 (with full pension) introduced if only temporarily so that a more normal flow of young academics could be reintroduced. I believe the overall cost would be tolerable.

●See page 552 for the legal position on short term contracts in the UK

news in brief

US scientists increase estimates of fluorocarbon survival rates: Research workers with the National Oceanic and Atmospheric Administration have used air samples taken from a global network of monitoring stations to conclude that flourocarbons emitted from aerosols and other sources survive for at least 30 years in the lower atmosphere, considerably longer than previous estimates of the minimum lifetime. The scientists say that the longer periods predicted when their sample data were applied to theoretical models of fluorocarbon behaviour "indicate that fluorocarbons do not undergo significant reductions in the lower atmosphere due to rain-out, fall-out, or chemical reactions, and thus are free to diffuse up into the stratosphere relatively undiminished".

However the scientists also found that the concentration of nitrous oxide fluctuates in the atmosphere, rather than demonstrating any steady growth. From this they have concluded that the potential threat to the ozone layer from gas given off as the result of massive applications of nitrogen fertiliser may not be as great as some have feared. "We had expected to see a steady upward trend in this chemical, corresponding with large increase in the nitrogen fertiliser, but no such trend was observed", said Mr Walter D Komhyr of NOAA's Geophysical Monitoring for Climate Change Programme in Boulder, Colorado.

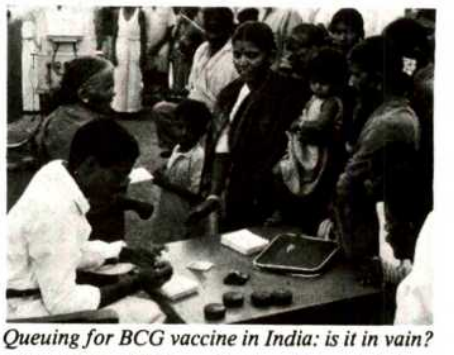
US refuses to lift ban on pesticide: Despite pleas from representatives of southern states that the health hazards of the fire ant outweighed the hazards of a pesticide, Mirex, capable of controlling the ants, the US House of Representative last week refused to lift a ban imposed on the pesticide in 1977 when it was suspected of causing cancer. Some delegates argued that the known dangers of the ant, which invades the homes of the poor and can inflict a potentially dangerous bite, should take precedence over the unknown hazards of cancer. But they were outvoted by those who felt the need, in the words of representative E de la Garza of Texas, "to take the road of caution and not risk irreparable damage to people and the land".

At the same time, the House voted by 278 votes to 121 to give Congress the power to veto future pesticide regulations issued by the Environmental Protection Agency, although this has not been agreed by the Senate and is opposed by the White House.

MRC proposes less job security for researchers: The UK Medical Research Council has proposed eliminating its "tenure track" and will try to make all tenured jobs competitive on the open market. Under the proposed system a new researcher would be hired on a five year contract with the possibility for a three year renewal but there would be no automatic tenure procedure — individual researchers would compete with outsiders for the tenured jobs that became available. The Association of University Teachers, the trade union that represents the researchers is opposing the move. The present arrangement has five-year limited term contracts with a 75% chance of tenure at the end. It was opposed by many researchers when it was introduced in 1974 as a redundancy procedure that "permitted the MRC to lay off 25% of its annual cohort every year". The MRC was "not prepared to comment at the present time" on the new proposal.

University of Grenoble spectrometry lab occupied: The laboratory of physical spectrometry at the University of Grenoble has been occupied since 14 November by 15 technicians protesting a reduction in their pay of between 20% and 32%. The technicians, all highly skilled with 15-20 years of research experience, fell victim to a complex administrative decision on 1 November which prevents them from receiving pay from two sources — the university and the government — an arrangement that has existed for 20 years. The laboratory employs 130 researchers, technicians and administrators all of whom are in support of the technicians. The lab's 85 physicists have lobbied the university administration and last Friday a one day full scale occupation of the administration building was held in an attempt to bring about negotiations. The matter is now under discussion.

New TB vaccine needed in India: The BCG vaccine used world-wide against tuberculosis has been found to be ineffective in cases of lung TB in India. This is the finding of a major seven year study carried out by the Indian Council of Medical Research in conjunction with the World Health Organisation. The study has found, however, that the vaccine still affords protection against brain and bone TB. The revelations come at a time when the incidence of TB is showing a sharp upturn and the implications of the study extend to other Third World countries. There are an estimated 10 million TB victims in India with 2 million cases known to be infectious. Along with other Third World countries India is now confronted with the complex problem of evolving an alternative to the BCG. *B. Radhakrishna Rao.*



Queuing for BCG vaccine in India: is it in vain?

UK national anti-nuclear campaign launched: The first nationally coordinated extra-parliamentary anti-nuclear campaign in Europe was launched at a recent meeting of 600 activists in London. UK groups expected to ratify the campaign's constitution represent the full range of UK political opinion and include the Conservation Society, the Ecology Party, The Young Liberals, the Socialist Environment and Resources Association, the Socialist Workers Party, the National Union of Miners and local groups throughout the country. The campaign aims to reduce energy demand through conservation measures, to replace nuclear power with alternative energies and to guarantee employment during the changeover. Convenor Tony Webb said that the campaign would also focus on the witholding of information by the nuclear industry. "We have been calling for many months for the safety reports on both the AGR and the proposed PWR without success. Secrecy in these matters only confirms the widespread belief that the technology has not been designed for safety and is in fact extremely hazardous."

In the meantime a new UK study (*Decision Making for Energy Futures, A Case Study of the Windscale Inquiry*, Macmillan) has been published this week that is sharply critical of government policy on information. Calling for a freedom of information act authors argue that access to information, "a fundamental axiom of democracy", is a concept "alien to much twentieth century practice in the United Kingdom". And in a related development, the UK has refused to pay its share of £6m to fund an EEC research project to study the controlled meltdown of a Harrisburg type reactor at the Supersara pressurised water reactor in Northern Italy. Government officials say that the cost of the experiment does not justify the probable results.

US nuclear industry wins 1979 Doublespeak Award: The US National Council of Teachers of English has awarded its 1979 Doublespeak Award for the most appalling public use of the English language to the nuclear power group at Three Mile Island. Out-distancing a record number of nominees for the prize, the officials at Three Mile Island were cited for inventing "a whole lexicon of jargon and euphemisms" that played down the danger. Explosion became an "energetic disassembly", a fire "rapid oxidation" and a reactor accident a "normal aberration".

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Berufsverbote continues in spite of resolutions

West German campaigners against Berufsverbot are planning to mark Human Rights Day, 10 December, with a petition to the Federal Chancellor, asking that the promised "liberalisation" of employment policy be implemented. **Vera Rich** talked to a leading member of the campaign about the effect of Berufsverbot on academic life.

BERUFSVERBOT is the denial of professional employment in the government service to persons of allegedly left-wing views. It affects West German universities and academic institutions because the funding of such bodies by the state means that their staff are "state employees" — in the same category as train drivers and postal clerks. Nevertheless, — and rather strangely in view of Germany's previous experience of political disruption of academic life during the 1930s — it was only relatively late in the anti-Berufsverbot campaign that certain universities and academic bodies began to take a stand on Berufsverbot.

> ❛ the social-liberals are convinced that Berufsverbote must end — but it still hasn't stopped ❜

In recent months, resolutions condemning Berufsverbot as a threat to academic liberty have been passed by the senates of the Universities of Oldenburg, Bremen, and Bielefeld, and the Deutsche Gesellschaft für Sociologie, amongst others. In the words of the Bielefeld protest (17 January 1979), the "autonomy of scientific work guaranteed by the German Federal Constitution forbids any attempt to exploit the scientist's status as a civil servant, to supervise his scientific activity by means of methods which are alien to science, or to derive restrictions on scientific activity from the loyalty and allegiance owed by the civil servant". The present practice of "screening and punishment for political beliefs", said the senate, "contradicts the liberal and democratic principles of our constitution," and, together with the administrative decrees on which it is based, "must be declared null and void".

Nevertheless, within a few weeks, Jan Priewe, selected by Bielefeld University as assistant professor of sociology was subjected to an *Anhorungdverfahren* (interrogation process) based on alleged left-wing activities more than five years previously.

Dr Andreas Dress, a leading campaigner against academic Berufsverbot, told *Nature* that it was precisely this screening process which was to have been "liberalised", but that "in fact almost nothing has changed. What we wanted was an end to the involvement of the *Verfassungsschutz* (Security Service) in job placements". In his own *Land* (province) of North-Rhine-Westphalia, for example, applicants have to fill in a form giving all their addresses for the last five years. This list then goes on to the *Verfassungschutz* computer, "proving," he said, "that the state government does not trust its citizens. But in a democracy, the state exists for the good of the citizens — and the Federal Republic of Germany is a democracy! This convinced the social-liberals that the practice must end".

"But it hasn't stopped," he added, "which is why we have organised the current protest. And even if it did officially stop, the personnel section could always pass on addresses to the *Verfassungschutz*. Still, it would be something if the practice were stopped at least officially."

"We are not arguing about genuine security checks," he went on. "We accept that they have to exist. But security can go too far. One politician, for example, justified Berufsverbot on the railways by noting that, during the Nazi occupation of France, French resistance railway workers had been able to half the whole country's rail traffic!"

Within the universities, Dr Dress explained, there is no over-all policy of screening one faculty more closely than another — sociologists, say, more than mathematicians. Some institutions, however, seem more at risk than others. Thus in the Max-Planck Institute of Biology, Chemistry and Physics in Göttingen, screening procedures do not seem to have been applied.

On the other hand, certain universities — Bielefeld included — seem to have more than their fair share of Berufsverbot cases. This, explained Dr Dress, is a result of the more liberal and open-minded attitude of such universities to innovations in course structure and content. Potential victims of Berufsverbot, he said, are less likely to apply to highly traditional establishments, either because they feel they would have no chance of acceptance, or simply because that particular milieu does not attract them. He cited the example of Horst Eckhart Gross, a victim of Berufsverbot screening when he applied for a post at the University of Oldenburg. Previously, Gross had directed a Bielefeld research project on the non-academic job deployment of mathematicians.

Of university cases of Berufsverbot, said Dr Dress, some 80% related to alleged past or present membership of the Communist Party or the "Spartakus" Marxist student union — both, he stressed, legal organisations. Some 10% of cases, mostly in Bavaria, relate to left-wing Social Democrats, and the remainder to miscellaneous left-wing groups. Formally, Berufsverbot should also cover far-right extremists, but according to Dr Dress such cases virtually never occur.

To date, Dr Dress knows of some 4,000 Berufsverbot interogation procedures. These involve prolonged waiting, and, in some of the German *Länder*, such as Bavaria, those undergoing investigation are not allowed to use the services of a lawyer, while in North-Rhine-Westphalia, the right to legal assistance was won only after "a long struggle". Out of the 4,000 cases some 3,000 ended either with reinstatement in the desired post, or else in the applicant abandoning his claim and taking a job in the private sector. Some 1,000 clear cases of Berufsverbot remain on the campaigners' files. However, Dr Dress went on, academics have no "private sector" to go to.

The anti-Berufsverbot campaign assesses all alleged cases very carefully. Problems of student activists expelled for poor academic performance cannot strictly speaking be considered as Berufsverbot. Nor can the recent case — described by *Die Welt* as "grotesque" — of a biologist charged under the German Official Secrets Act for having given his East German brother-in-law an offprint of one of his openly published papers — this was a technical breach of the law. To the anti-Berufsverbot campaigners, it is important that all provocation be seen to come from the official side. "We are really only interested", said Dr Dress, "in cases where the authorities state in writing that the person is banned from employment on political grounds only. Then we can fight."

> ❛ over 70 million DM has been spent in counter-propaganda. ❜

The authorities, incidentally are not slow to take counter-measures against the damage done to their image abroad by the anti-Berufsverbot campaign. Dr Dress recalled that in April of this year, the Christian Democrats tabled a question in the Federal Parliament, demanding to know what the ruling Socialist-Liberal coalition had done to neutralise the influence of the campaigners. To date, according to the official reply, over 70 million DM has been spent in counter-propaganda, mostly among journalists in foreign countries. It is perhaps for this reason, Dr Dress concluded, that initiatives from the campaigners, such as the establishment last February of the "Heinrich Heine Fund" for victims of Berufsverbot, receive little publicity abroad. □

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Brazil: running on alcohol

Brazil leads the world in the development of alcohol as a fuel for transport. But has it really found a way of appreciably reducing its dependence on oil? Maurice Bazin reports.

THE Brazilian government is plunging into a programme to convert all of its automotive transport to alcohol combustion within the next few years. The technical feasibility has already been proved, and several fleets of government vehicles already operate on pure ethanol.

The PROALCOOL programme was launched in 1975 in response to the world wide increase in oil prices, in an effort to contain Brazil's record US $45 billion foreign debt. Domestic oil production accounts for only 16% of consumption, the rest being imported — almost exclusively from the Middle East — and refined in Brazil.

Ethanol (ethyl alcohol) is produced in Brazil from sugar extracted from sugar cane. Extensive cane fields dating back to colonial days cover the coastal north-east of the country; but these fields have very low yields. The landowners, referred to as 'colonels', reign over their peasants through armed 'captains'. Salaries remain below US $2 a day and never cover the peasant's debt at the general store - which is owned by the colonel. Nowadays, 80% of sugar production comes from the more developed state of São Paulo to the south where agribusiness organisation prevails.

Alcohol production could currently satisfy one-third of Brazil's automotive needs if all private cars were run on pure alcohol. Brazil has in fact been using 'gasohol', petrol mixed with alcohol, since 1931, when the addition of 5% alcohol to petrol was made compulsory. Today, the mixture is not uniform throughout the country, being higher in the states with higher alcohol production, and reaching a maximum of 20% in São Paulo. Until 1975, the mixing proportions were not dictated by practical considerations, alcohol being dumped into petrol as an economic buffer for excess sugar production when international sugar prices dropped.

Some economic experts are apprehensive that the PROALCOOL programme will lose impetus if world sugar prices start rising again. They insist that to make the technological option irreversible and independent of the fluctuations of the international commodity market, new distilleries should be built which operate directly from raw cane, bypassing sugar production and storage. The Institute of Sugar and Alcohol (ISA) which administers PROALCOOL has indeed approved the construction of 87 such new 'autonomous' distilleries. But it also approved the construction of 139 standard distilleries, which will double the present number of distilleries operating from sugar as an intermediary. This decision leaves some doubts as to the earnestness of the government's commitment to a fully independent alcohol program.

Alcohol production has increased dramatically in the past few years. In 1976-77, 600 million litres were produced; in 1977-78 production reached 1.5 billion litres and has risen to over 3.5 billion litres this year. The ISA announced that "the sum of the already operating installations and of those projects already approved or under consideration should produce over 5 billion litres of alcohol. We can be assured of an overall production of 4 billion litres in 1980, which will guarantee the 20% level for the mixture of alcohol with gasoline". The goal for 1985 is 10.5 billion litres.

One problem with this rapid increase in production is storage. Due to a miscalculation by ISA, storage capacity was found to be inadequate this spring. As a result, distilleries in São Paulo are threatened with having to halt operations, and Petrobras, the national company controlling the petroleum industry, is hastily renting out its petrol storage facilities to store the alcohol presently being produced. Furthermore, the government authorised the export of 500 million litres of alcohol, at a price double that of imported oil.

Up to 20% ethyl alcohol can be mixed with petrol without any engine modification, and the optimum mixture, which minimises fuel consumption , occurs with 15% alcohol. Brazil pioneered the study of alcohol engines in the 1920s. For the past ten years, the Brazilian Air Force Technical Institute has investigated the use of straight ethanol in modified automobile engines, and each of the foreign companies that control the Brazilian automobile industry (Volkswagen, Fiat, Ford and Chrysler) has developed new alcohol-powered engines for passenger cars.

Existing petrol engines are already routinely modified in São Paulo for the Volkswagen 'beetle' and the Brasilia model. The changes are few, and currently cost about US $350 per car. The work is done in standard engine overhaul shops approved by the Ministry of Industry and Commerce. Since alcohol has a higher vaporisation temperature than petrol, the incoming air must be preheated to at least 70°C to insure complete fuel vaporisation. This is usually achieved by passing the air through a jacket around the exhaust system, but other schemes using the engine oil in a preheating jacket are also being investigated. These engines will not start when cold, so a small auxiliary petrol reservoir must be installed to start the engine on pure petrol.



Wania Sandis Dell 'Amico

Refuelling on 'gasohol', a petrol-alcohol mixture used in Brazil since 1931

As well as testing the use of straight ethyl alcohol in modified automobile engines, the Air Force Technical Centre has engaged in a vast public opinion campaign. It organised several public demonstrations two years ago including a 'National Integration Tour' during which three test vehicles covered 8000 km in tropical regions. Car races, normally prohibited in Brazil, have been specially authorised to test new ethanol-powered models. Brazilian-built Fiats and Volkswagen sedans (Passat) were run continuously for two weeks, covering more than 100,000 km without problems. In these and other competitions, racing drivers showed enthusiasm for the engines, finding they could reach higher mean speeds than were possible with the equivalent petrol-powered models. They discovered experimentally that the best performances were obtained with an engine temperature 10–15°C *cooler* than the gasoline engines. This developed a higher torque, along with improved engine durability. The only technical problem not yet fully tested is corrosion.

No systematic study of pollution caused by ethanol combustion has been undertaken by official agencies. But it is clear that alcohol is both lead-free and sulphur-free to start with; while the lower engine operating temperature is liable to produce less polluting compounds.

But PROALCOOL pollution problems appear dramatically at the source. Distilleries are always built beside rivers,

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into which they dump the stillage, or 'bottom slops', coming out of the first distillation column at a rate 12 times (by volume) that of the alcohol produced. The total output of stillage in Brazil is equivalent in biological oxygen demand to the untreated sewage produced by a city of 200 million inhabitants. Studies at the Copersucar research centre in São Paulo show that the high potassium content of stillage is responsible for killing both fish and aquatic flora. Although stillage can be converted into fertilizer or simply spread back on the fields after 'lagooing' for a while, this is hardly ever practised in Brazil, where distillery owners are not bound by any environmental regulation.

The early political commitment to use alcohol as a fuel has led Brazil to a definite technical lead. National pride ran high in October when Frank Press, President Carter's adviser on Science and Technology, who was touring Brazil in an alcohol-powered car, declared: "This is not an aid mission. Our visit is to initiate a mutually beneficial cooperation. Brazil is ahead of the whole world in the area of alcohol fuel, and the United States is interested in the PROALCOOL programme." At the same time the Brazilian Minister of Industry and Commerce, accompanied by the president of the Alcohol and Sugar Institute, was in Bagdad to sign an agreement of technical cooperation with Iraq which includes an offer of Brazilian technical know-how.

A recurrent theme among planners and politicians alike is the drive for technological independence through the use of those Brazilian technologies which are best established. "We have many raw materials from which we can extract alcohol," says Jair Carlos Melo, professor of Energy Resources at the Federal University of Minas Gerais in Belo Horizonte. "We should not stimulate national competition between them but rather select those which are immediately usable. This attitude will avoid our facing in the future the same problems with PROALCOOL which we now face with the nuclear energy programme: finding ourselves dependent upon foreign technology." This implies emphasis upon sugar cane, however, and reinforces the conservative agrarian social structure in the Brazilian countryside, and increases the power of the sugar 'colonels' and the owners of mills and distilleries.

Plans to diversify the sources of ethanol are being made, however, at least on an experimental basis, in the Ministry of Agriculture. Ethanol could be produced by the fermentation of wood. The cost of a pilot plant with a capacity of 100,000 litres per day is officially estimated at US $20 million, "which is less than our daily expenditure for oil imports," says the Ministry's general secretary. But the plan is far from complete; the choice of a technology best-adapted to Brazilian wood will be made by a semi-private company still to be incorporated.

The other highly promising alternative for alcohol production is 'mandioca', the plant from which comes tapioca. Mandioca has the great advantage over sugar cane that it can be grown in poor soil. Traditionally it has not been grown on large plantations by powerful landowners, like cane, but has remained the poor man's foodstuff. Only one experimental distillery operating from mandioca exists at present in Brazil. It was built by Petrobras in the State of Minas Gerais as a demonstration plant on an industrial scale, and produces 60,000 litres per day. Ten other projects have been approved by the government, several to be run by universities. One is also an attempt to decentralise energy production: the alcohol from mandioca will be used to run a turbine and produce electricity in the northern state of Ceará. Without specific government subsidies, it appears that private entrepreneurs do not dare as yet to invest in the construction of mandioca distilleries.

The development plan for the PROALCOOL programme relies upon the investment of US $1.75 billion in five years. So far, a loan of $1 billion has been obtained in London but the Brazilian Central Bank will free only $350 million per year for the programme, keeping the balance in reserve. That sum is at present almost entirely devoted to financing new distilleries and modernising existing ones.

Some still have doubts

Vocal critics of the programme are found among economists and sociologists concerned with overall agricultural development. They point out that with presently existing technology sugar will be the only prime material for the production of alcohol during the next five years, and the present area under sugar cane must be more than doubled to meet the goals of the programme. Sugar cane demands fertile terrain and often special irrigation. Unless there is overall planning and coordination of agricultural production, easily available financial incentives for sugar cane planting will increase the social imbalance of Brazilian society. Sugar cane, now 'alcohol cane', could easily displace much-needed food crops and the regional imbalance and social tensions already characteristic of the Brazilian countryside will be exacerbated.

Trades unions have taken advantage of the combining liberalisation of the military regime to promote debates about the government's options for development. The Federation of Agricultural Workers of the State of São Paulo and the Distillery and Refinery Worker's Union are conducting "forums of the unconsulted", warning that the extension of the sugar cane agro-industry will "increase the inequalities in land use and ownership, in income distribution and in the distribution of the benefits of progress".

Even some state governments reject the grandiose schemes which the federal government has planned for them. Thus the Agricultural Secretary of the state of Parana, immediate neighbour of São Paulo to the south, plans to authorise the construction of only 20 new distilleries rather than the 40 of the federal programme. "I reject the megalomania of the State of São Paulo", says Reinhold Stephanes, announcing that production per distillery would be limited to 120,000 litres of alcohol per day instead of 400,000 as in São Paulo. Five thousand hectares are necessary to produce 120,000 litres per day, and the regulations established in Parana demand that 25,000 hectares be reserved for other crops in districts where distilleries will be located.

The greatest doubt about the whole PROALCOOL programme concerns its ability to contribute to a real reduction in the outflow of dollars, or even to economise upon oil imports. During recent seminars throughout Brazil to consider the country's energy options, several engineering consulting firms have pointed out that the substitution of petrol fuel must be considered in a much broader context than has been done so far. Existing refineries produce fixed, and roughly equal, proportions of petrol, gasoil and diesel fuel. These proportions correspond closely to the consumption of each product. Alcohol, however, would substitute for only one of them. Refining schemes are quite rigid and any modification in the proportions of the final products would require enormous investments on the part of Petrobras.

To reduce its imports of crude oil, Brazil must therefore "wage a global struggle against the barrel", says Sergio Trindade, director of Promon's Technology Centre, a large consulting firm in Rio de Janeiro. In his presentations at national seminars, Trindade has pointed out that Brazil is at present totally dependent upon diesel fuel for its transport; more than 90% of goods being transported by lorry. Maritime transport accounts for less than 3%, although the country has a very long coastline and excellent harvours. The latter have traditionally been used exclusively for exporting cash crops (sugar, cocoa, and coffee) rather than for internal commerce. To reduce seriously Brazil's dependence on imported crude oil, control external debts and achieve what the government calls 'strategic independence', all three components of the barrel must be reduced at the same time. This means that the whole energy consumption pattern of the country must be altered. Small steps are not enough. Trindade would like to see an 'alcohols' programme get under way as part of national energy sources evaluation effort. This is the dominant theme of numerous discussions taking place at present throughout the country with the participation of ministers and scientists to eleborate a new 'Brazilian energy model'. □

Short term contracts: the legal status in Britain

British law on fixed term contracts is unclear. Basic questions, such as 'what is a fixed term contract?' and 'how effective is a waiver clause?' have still to receive satisfactory answers. **Julian Fulbrook** of the London School of Economics, traces the recent legal wrangles.

THE shabby treatment of short term contract workers continues to be a matter of concern. Although in the scientific and university context the exploitation of workers has never reached the depths of casual work problems experienced in the docks or building industry, the existing legal position is far from satisfactory.

Various estimates put the numbers involved at around 10,000 postdoctoral and other short term contract researchers in the United Kingdom. Of course, a strictly legal analysis ignores the wider general debate on tenure and temporary posts. And no one can ignore the desperate plight of highly-trained individuals who have hardly settled into a new position when they are required to forage for the next project, knowing that a new generation of younger researchers will be cheaper to employ. Mr Justice Kilner-Brown remarked in a recent case that the legal profession contains a similar log-jam between pupillage and a tenancy in chambers, but most would-be barristers are quickly snapped up by industry and the Civil Service. No such immediate alternative presents itself to scientists.

In considering the legal position of short term contract workers, it is important to note the threshold qualifications for Unfair Dismissal and Redundancy. The minimum period before a claim can be issued is now 52 weeks of employment for Unfair Dismissal and two years for Redundancy. It is therefore commonplace for a research worker to be fully in employment for a number of years, but at wholly different establishments for periods of months, and never build up an entitlement. The position is perhaps most acute for women who would wish to take up state maternity pay but are unable to stay in one institution for the requisite two years.

The usual legal questions, however, revolve around the nature of dismissal by expiry of a fixed term contract, and the potential waiver by a short term contract worker of his job security entitlements.

It would be an obvious loophole if an employer could evade the statutory protection given to employees against Unfair Dismissal by giving an endless series of short term contracts. The law since 1963, on the whole range of employment protection legislation, has therefore laid down certain rules to stop up this loophole. Unfortunately it can do little to help the worker who, through force of economic necessity, has to move through a variety of employments; however, if his employers are 'associated', the continuity of rights may be maintained. Essentially, in relation to Unfair Dismissal or Redundancy, employment protection exists when any fixed term contract, of whatever length, comes to an end without renewal. This preliminary hurdle passed, the law then turns to the question of whether non-renewal was fair or unfair.

The waiver provision is more complex. To achieve an effective waiver, the contract of employment must be for a fixed term of two years or more. The statute insists on four requirements before the waiver ousts the jurisdiction of an industrial tribunal:

- The waiver must be in writing, in contrast to the general rules on employment law which give binding effect to oral terms.
- The written agreement must be made before the expiry of the fixed term contract
- The dismissal itself must consist only of the expiry of the fixed term contract without its being renewed. For example, if the worker is accused of misconduct or 'not being up to the job', then his remedy is under Unfair Dismissal and the waiver is not a bar to his claim.
- The final requirement has been the basis of confused litigation: the contract must be for a *fixed term* of two years or more.

But what precisely is a fixed term? The legislation is not entirely helpful. Section 49(4) of the Employment Protection Consolidation Act 1978, which gives an employee statutory minimum periods of notice, uses the concept of a *term certain,* whereas all the other statutory provisions refer to a *fixed term.* The significance of this is unclear, but in any event it has been brushed aside in the recent leading case of the Court of Appeal in *Dixon v. British Broadcasting Corporation* [1979] Industrial Cases Reports, page 281.

To understand the importance of the *Dixon* case it is necessary to recall the earlier decision of the Court of Appeal in *BBC v. Ioannou* [1975] ICR 267. Here Lord Denning had said that a fixed term was "one that cannot be unfixed by notice". In other words if there was an express term to allow either side to part company amicably before the stated term, then the contract was no longer for a fixed term. As notice provisions are practically universal in contracts of employment this had the effect of greatly reducing the scope of the exclusion provision. This may have been desirable in some respects, but the case had disastrous consequences overall for the short term contract worker. As a notice provision rendered a 'fixed term contract' simply an ordinary contract, the worker could not claim that he had been dismissed through the expiry of a fixed term. And it would not be possible to qualify for Unfair Dismissal or Redundancy under the head of an ordinary straightforward dismissal, or even under a constructive dismissal, because of course in reality the contract had terminated by the 'effluxion of time'. The short term worker fell into a limbo.

Various Industrial Tribunals and the Employment Appeal Tribunal tried valiantly to avoid this injustice. Now in *Dixon,* the Court of Appeal declares that they were wrong in *Ioannou.* Lord Denning admits engagingly that he admired the ingenuity by which the tribunals were evading his previous definition of a fixed term to achieve a 'sensible result'. He noted the criticisms of many commentators that an employer could always evade the Act by inserting a simple clause 'determinable by one week's notice', and he declared that this could not have been the intention of Parliament.

The details of *Dixon* were that the BBC employed two men in Manchester as 'house services attendants'; Lord Denning thought that this was "a grandiloquent way of describing a commissionaire or a porter". Both were employed under fixed term contracts which, after several extensions, were due to expire "unless previously determined by one week's prior notice in writing on either side". Each employee had signed the extensions which always included the notice provisions. The final fixed term was for only four weeks, but the BBC disputed strenuously that it was for a 'fixed term' on the basis of *Ioannou.* To illustrate the see-saw nature of litigation, it should be noted that the Industrial Tribunal decision followed *Ioannou,* the Employment Appeal Tribunal allowed an appeal distinguishing *Ioannou,* and the Court of Appeal dismissed the BBC's appeal and overruled part of *Ioannou.*

The upshot of the Court of Appeal decision is that a fixed term contract may nonetheless have within it the possibility of termination by notice and yet remain a fixed term contract in law. *Dixon* has therefore resuscitated a number of Originating Applications which, having been earlier knocked out on jurisdictional grounds, are now able to proceed before the Industrial Tribunals.

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Paradoxically, the Court of Appeal has increased the importance of the waiver provision. The original rationale behind the redundancy exclusion was explained by Professor Grunfeld, Professor of Law, London School of Economics, as being that "an employee taken on for a fixed period knows from the beginning that his job ends on a certain date . . . The fixed term employee knows what is to come and is therefore in a position to plan ahead for himself". But such a view cannot tenably be expanded to cover an exclusion from Unfair Dismissal. The law at present provides for fair dismissal in relation to lacklustre performance in a probationary period, and the continuance of the arbitrary two-year fixed term rule operates merely as a loophole for an employer to evade the Act.

It is unrealistic to imagine that research workers can individually refuse the imposition of a waiver when there may be hundreds of other candidates willing to take up the post. Of course the practical solution is through collective negotiation to abolish the waiver, but given the lack of unionisation and the ease of importing new research workers, this can only be a long-term aim. Meanwhile, even where a waiver is not utilised, employers mould their contracts to the law; a favourite device is the 21-month contract, which creates the problem of finance over the summer before the start of a new academic post.

An important question arises over multiple contracts: is it possible to amalgamate the cumulative period of a series of fixed term contracts to achieve a fixed term of two years? In *Ioannou* the Court of Appeal, in a part of the judgement that has not been overturned, held that it is only the 'final' contract which has to be considered. Only where this final contract is for a fixed term of two years or more can there be a valid waiver. Although lawyers would normally draw a distinction here between a 'renewal' or a 're-engagement', Lord Denning regarded this as "too fine a distinction for ordinary mortals". His approach was followed in the Employment Appeal Tribunal decision of *Open University v. Triesman* [1978] ICR 524, where the employee was offered a post as a lecturer for a fixed period of 18 months.

However, this 'straightforward' view has strange consequences. If an employee starts with a 21-month fixed term contract and is therefore unable to make a waiver, but then goes on to a two year renewal with a waiver, he will be unable to claim a redundancy payment. The first period will be blotted out, and no claim is possible.

This and many other legal puzzles leave the law in an uncertain state. There is still the faint chance of an appeal in *Dixon* to the House of Lords changing the law all over again, or even the possibility of major legislative change by the present government which has as yet given no precise indications as to the parameters of planned amending statutes. The only certainty is that the present law is in urgent need of an overhaul to resolve the waiver dilemma. Although this of course will do nothing to change the demand for short term contract workers, it may prevent some of the anxieties suffered by them. □

Carnivorous practices



THOMAS H. JUKES

THE subject of meat eating is one that entrances gourmets, repels vegetarians, and attracts anthropologists. It has fascinated physiologists ever since the experiments on the fistulated stomach of Alexis St Martin by William Beaumont.

Something new happened recently: it started in a restaurant in Washington that was serving Pennsylvania rattlesnake meat to its patrons, thus reversing the balance of nature in which snakes bite hominids. Incredible as this would have seemed even as recently as 30 years ago, this venomous serpent is protected as an endangered species. An employee of the US Fish and Wildlife Service wrote a letter to the restauranteur, tactfully pointing out the act of trespass, whereupon the restaurant switched to Texas rattlesnakes for serving to its herpetophagic patrons.

All seemed well at first — except for the Texas snakes — but perhaps a conservationist intervention will soon be organised on their behalf. However, the news leaked out, whereupon the offending civil servant was fired by order of the Secretary of the Interior. A thunderous protest against the firing was mounted by lovers of Pennsylvania rattlesnakes and haters of dictatorial cabinet members, so that the Secretary hastily re-hired the snake-protector, bowing to the uproar from Congress, and from environmentalists, who are still seething over the betrayal of the snail darter.

It was time, I thought, for some general comments on meat eating, a mode of sustenance that involves a complex pattern of approvals and taboos. Probably none of us is without prejudice, distaste or downright rejection for some forms of meat that are relished by ethnic groups to which we do not belong. Nutritionally speaking, the proteins of various meats are quite similar in amino acid content, and are superior in this respect to vegetable proteins, but this does not affect their acceptance or rejection.

Avoidance of beef in India is one of the best-known taboos. One theory is that it originated when Brahmins renounced cattle sacrifice to improve their position in political rivalry with the Buddhists. The shunning of pork is centered in the Middle East, and the feelings of rejection can be intense enough that martyrs have chosen death rather than submitting to being forced to eat pork.

It is often firmly asserted that the Jewish ban on pork originated as a protective measure against trichinosis that was based on intuitive and shrewd trial-and-error observations. This is contradicted by Simoons, F.J. *Eat Not This Flesh* (University of Wisconsin Press, Madison, 1961), who points out that *Trichinella* was unknown until the nineteenth century and was considered to be a harmless organism for 25 years after its discovery. It is unlikely that spoilage of pork by heat is the reason for the rejection. Temperatures in the Middle East are no higher than in many countries such as Southeast Asia, where pork has been a prized food for many centuries.

The evaluation of other possible reasons for the ban, such as distaste for the uncleanliness of pigs, is complicated by the fact that Jewish laws also banned the eating of 30 kinds of animal including some comparatively tidy creatures, such as snakes. Chickens, however, are on the approved menu, even though their eating habits are at least as unsanitary as those of pigs. Americans and Western Europeans, who avidly swallow live oysters, would recoil from eating dog, which is relished in Central Africa, China and Southeast Asia.

The eating of frogs and snails was formerly used to whip up feelings of xenophobic prejudice in England against the French. In riposte, the English were called 'limeys', but it seems difficult to arouse antipathies based on the consumption of fruit, and, in any case, limes prevent scurvy.

The new approach to the avoidance of foods, including meat, and especially beef, is based on arousing the fear of cancer. I shall discuss this, and more meat-eating matters next time.

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correspondence

Researchers Information Exchange

SIR, — Neither your editorial *Time for a policy on scientists' jobs* (1 November, page 1) nor the report by Joe Schwartz on *Portrait of the out-of-work scientist* (1 November, page 7) deserve to pass unnoticed. It is high time that the maximum of serious attention was given to the undoubted crisis which has developed in medical research and in research in other fields. The case of Dr Ann Simmonds and the Purine Laboratory at Guy's Hospital is only the tip of an uncompromising iceberg, that in the not too distant future is certain to confirm at last the United Kingdom's position as a third rate economic and scientific power, with a complement of first rate scientists, and scientific opportunities going to waste. Now is the time to ask "How long is it?" until that future comes to pass.

Some months ago the Association of Researchers in Medical Science committee received a very stony answer from the Department of Education and Science when they entered a plea to the Secretary of State to initiate a major inquiry into the scientific research sector (in a time of constraint and crisis), with particular reference to the conditions of employment of scientists and support staffs. In the meantime researchers' organisations, trades unions and campus opinion has been mobilised to publicise the plight of many researchers and scientists (in all disciplines).

The need to act in order to protect and develop conditions of work now seems a necessary distraction that research workers must pursue for their own survival and for our scientific future.

Government complacency and indifference in these matters is no longer defensible. In fact, some gesture from that quarter might redeem the situation before it gets really out of hand.

May I, through your columns, appeal to researchers and scientists to press energetically for a major review of the whole research and development sector, by the combined efforts of government, scientific and professional agencies. If our current economic straits do not allow the full panoply of review, at least a start must be made to identify problems and solutions so that scientific research policy can once again be adequate and informed. If action is not initiated soon we must accept irretrievable losses and delays to scientific progress, and technological and social applications. Can we bear to estimate our regret in five or ten years time by not having taken a positive stance now?

In the immediate future researchers and scientists must take steps to collaborate and coordinate their efforts in what is likely to become a bitter political battle if no firm action to seek just remedies is taken.

To this end a group of researchers (including myself and Dr Simmonds) have already established and gathered support for a *Researchers Information Exchange* to collect, analyse and distribute information on all aspects of research and development, relating to employment and conditions of work, and in the broader area of organisation, cooperation, funding, planning and policy-making. A recent meeting of RIE participants at Leicester University voiced the need for unified policies in the field of research, and it is to this end that readers and colleagues are urged to lend their support to this venture as a first stage in self-help to ensure the continued existence of a viable scientific community in this country.

Yours faithfully,

STEPHEN ROBERTS

Loughborough University, Leicestershire, UK.

Life-time of research for minority only

SIR, — You are right to say (1 November, page 1) that hard thinking is needed about the nature of scientific careers. As I have argued elsewhere, however (*New Scientist*, 17 May 1973) one of the foundations for such thinking must be that research alone cannot provide a life-time career for any but a minority. Until this unpleasant truth is more widely recognised, we shall continue to have the waste and personal tragedy which you illustrate in the same issue as your leader.

Yours faithfully,

D.W. BUDWORTH

London W4, UK.

Alexander Lerner: a call for action

SIR, — We are extremely disturbed about the case of the distinguished Soviet scientist, Professor Alexander Lerner, who for eight years has been incurring the displeasure of the Soviet authorities because he applied for permission to emigrate to Israel. Professor Lerner was Director of the Department of Large Scale Systems at the Institute of Control Sciences, and among his publications is a well known text book, *Fundamentals of Cybernetics*, which has appeared in many languages.

Following his application for a visa he was: dismissed from his post and the Communist Party and forbidden to teach any students; expelled from the Editorial Board of the journal *Automation and Telemechanics*; removed from the public office of Chairman of the Scientific and Technical Committee of the University of Moscow. An attempt was also made to remove him from the post of local deputy Chairmanship of the Committee of the International Federation on Automation Administration which was rejected by the Federation.

After selling 40,000 copies, his book *The Beginning of Cybernetics* was taken out of production and his contract for this publication cancelled because of "mistakes in the manuscript", although a translation was published in many countries and it was recommended for the Competition of Best Books in 1967. The book *Optimal Heating of Metals* of which he was the scientific editor and one of the authors, was published in 1972 by the Metallurgy publishing house without his name being mentioned and without any reference to his works which are of fundamental value in this sphere. He has been prevented from attending conferences both within his own country and abroad.

Professor Lerner's daughter received a mathematics degree at the age of 15. In 1974 she and her husband were allowed to emigrate to Israel where they now live with their two children. Professor Lerner who is 66 years old has never seen his youngest grandchild.

We are endeavouring to ameliorate the fate of this man who is isolated from his scientific colleagues and his work. His many interests clearly lie within those fields covered by the International Federation of Automatic Control (IFAC). We hope that these international scientific bodies such as IFAC will show they have a clear duty to defend the rights of scientists working in their subject area. We therefore publicly call upon the IFAC Executive Council and National Member Organisations to consider seriously the fulfilment of their obligations in the case of Professor Lerner.

Yours faithfully,

PETER ARCHER DAVID SHEPPARD
for the parliamentary contingent

NEVILL MOTT A.H. VAN DEN HEUVEL
for the scientific contingent

The Lerner Circle, London NW3, UK.

European ingenuity in particle physics

SIR,—We write to correct some of the more grossly misleading impressions generated by the careless journalism of your article entitled "Can Yankee ingenuity keep US physics on the ball?" (1 November, page 2).

The reference to PEP as the machine "expected to be the first to discover the Z°" is obviously, from its context, a mistake for the antiproton-proton ($\bar{p}p$) collider now under construction at CERN. This machine is made possible by European ingenuity displayed in the development of beam 'cooling' techniques — stochastic cooling was invented at CERN and electron cooling at Novosibirsk in the USSR. The feasibility of cooling $\bar{p}$ beams has been demonstrated at CERN and the $\bar{p}p$ collider project quickly launched to use the SPS ring as a store of both $\bar{p}$ and p. The similar project at Fermilab which you mention is totally dependent upon these techniques.

The method proposed by Richter to achieve collisions of 40 to 50 GeV electrons and positrons is indeed relatively cheap as it depends upon the existence of an expensive two mile long accelerator at SLAC, built in the late 1960s and the only one of its kind in the world. We applaud the ingenuity of this proposal and hope that the very formidable technical problems can be overcome. However, even if successful, this device cannot attempt the fundamental research for which LEP is deliberately designed; that is, not only to make a complete study of Z° physics but to allow the most searching investigation of the weak, electromagnetic and strong interactions, performing vital tests of the gauge theory approach — such as the one due to Salam and Weinberg — which promises a unified understanding of these forces. These objectives require positron and electron energies approaching 100 GeV, not attainable at SLAC.

The last decade has been a time of dramatic advance in our knowledge and understanding of fundamental physical processes and we welcome the enterprise and ingenuity being exercised by physicists and engineers, both in Europe and the US, to bring nearer the crucial tests so eagerly awaited.

Yours faithfully,

R. J. CASHMORE
J. H. MULVEY

Department of Nuclear Physics, Oxford, UK.

news and views

Differential attack

from Robert W. Cahn

THE essentially empirical science of chemical etching, used as a routine tool by the material scientist to reveal the microstructure of mineral samples, has developed in some unexpectedly sophisticated ways. One is the recent development of a thin-film ultramicrosieve (Kitada *J. mater. Sci.* **14**, 2765; 1979), the latest in a line of filters whose unlikely origins lie in an observation made years ago that chemical etching could reveal the damage tracks left by the passage of nuclear fission fragments through thin mica sheets.

This story begins in 1961, when R.M. Walker and P.B. Price at the Central General Electric Research Laboratory in Schenectady discovered that these damage tracks were revealed by chemical etching which pierced minute, uniformly-sized holes through the mica. Other minerals were soon found to behave similarly, and other forms of radiation found sometimes to yield etchable tracks. Walker and Price, aided by R.L. Fleischer, over the next few years applied this differential etching method to many problems in geochronology, planetary physics (the study of meteorites in particular), palaeontology and nuclear physics. The field has become large enough to achieve that ultimate accolade, a journal of its own.

The early mica work came to the attention of S.H. Seal, a clinical cancer researcher at the Sloan-Kettering Institute who needed an ultrafilter to hold back cancer cells from suspension in blood; nothing he had tried would do the trick. Price's irradiated and etched mica foils were just right (the holes were all 3–4 μm across) but the foils were too fragile. At this point, Fleischer found that a polycarbonate polymer could be substituted for mica and had better mechanical properties. Thus began the manufacture of GE's 'Nucleopore' biological filters, which, among other uses have a role in cancer diagnosis. The filters were described by Fleischer, Price and Symes in *Science* (**143**, 249; 1964), and the steps in this weird progression from fission damage research to clinical instrumentation are traced, step by step, in that excellent survey, *Applied Science and Technological Progress* (313, NAS, Washington, 1967).

This case-history has evidently influenced subsequent research at GE. For instance, W. Desorbo and H.E. Cline, who were investigating directionally solidified eutectics, consisting of parallel metallic rods in a matrix, with a view to their use in jet engine turbine blades, found that differential etching left the rods protruding clear of the matrix. Porous metallic replicas made from such etched samples performed neatly as microsieves (*J. appl. Phys.* **41**, 2099; 1970).

Filters with holes a few microns across are considered coarse nowadays (too coarse, for example, for the isolation of airborne smokes), and a new approach has now shown how to make filters with submicron apertures. M. Kitada (*J. mater. Sci. op. cit.*) co-evaporated gold and germanium in vacuum onto sodium chloride crystals. Films 50–500 nm thick were evaporated, at or near the eutectic composition of 12 at% Ge. Germanium was deposited as round grains 20–250 nm across, depending on alloy composition and substrate temperature. The films were floated off in water and attacked by an iodated mixed-acid etchant, which selectively removed the more reactive germanium. Since the germanium grains grow in columnar fashion through the film thickness, removal of germanium leaves minute pores right through the residual gold films and the result is a thin-film ultramicrosieve.

Another form of selective attack which is not only technologically valuable but scientifically intriguing, is the vertical etching of silicon. This technique, which for several years has been quietly developed within the semiconductor industry, is now beginning to find applications in other scientific fields. An excellent account of the technique, its scientific basis and recent very various applications has just been published by D.L. Kendall (*A. Rev. Mater. Sci.* **9**, 373; 1979) who regards it as a 'whole new way of structuring and using a solid material'. The essential fact is that extremely narrow, deep grooves with depth-to-width ratios exceeding 100:1 can be etched in silicon. It is only necessary to start with a monocrystal slice accurately parallel to (110), and to prepare an oxide surface mask with narrow gaps precisely parallel to the (110)/(1$\bar{1}$1) intersection. Etching is very much faster in the [110] than in the [1$\bar{1}$1] direction, because the newly formed (1$\bar{1}$1) plane is quickly covered with an oxide film which severely hinders access of etchant (usually an aqueous solution of potassium hydroxide used at 80 °C), whereas other planes are not thus affected. Any divergence of the surface slit from correct alignment enhances the rate of etching of the sides of the groove.

Whereas it is easy to etch deep ultra-narrow grooves, it is not possible to etch round holes. A regular array of exact 5 μm-square holes has been fabricated by etching two populations of grooves, mutually inclined at 70°, from opposite sides of a (110) slice until they just meet (Bean, *IEEE Trans. ED-25*, 1185; 1978). Other uses include deeply etched solar energy absorbers (effectively thin-layer blackbodies), an elaborate high-area Si/SiO_2 capacitor only about 1 mm square, diffraction gratings efficient for high-order optical spectra and an infrared reflection polariser. The most subtle application is a device for ejecting ink-drops between closely spaced pairs of electrified p/n junctions to charge the drops, which can subsequently be electrically steered to form a printed image. This has cleared the way to computer-controlled printing without moving parts.

A variant on the sieve theme is a selective-ion device for separating ions such as Cu^+, which diffuse very fast in silicon. A thin-walled meander is formed in a silicon slice by etching offset parallel grooves from the two sides; copper will pass selectively through such a silicon membrane, much as hydrogen passes selectively through palladium. If the membrane is oxidised by anodising in hydrofluoric acid, the oxide has pores which can be as small as 1 nm in diameter. These can be used to separate molecules of different sizes: larger pores, for example, can serve as virus filters (Watanabe *et al. J. Electrochem. Soc.* **122**, 1351; 1975; Gregor & Gregor *Sci. Am.* **239**, 88; 1978). □

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Probing eukaryotic gene control

from Ron McKay

FOR two decades after the structure of double-stranded DNA had been determined, the difficulty of either establishing or manipulating a DNA sequence made even a descriptive approach to DNA-protein interaction difficult. The recent advances in DNA chemistry have been followed by improved methods of studying the association of DNA with protein and these techniques have provided valuable insights into such interactions as that between *lac* repressor and its operator DNA, the paradigm in this field, and those between ribosomal proteins and RNA. At a different level however, the most striking conclusion of a recent meeting* on this topic was that prokaryote-based rules of genetic organisation cannot completely explain eukaryote gene control.

A brown box

Any functional definition of a gene must include statements covering the mechanisms of transcription, translation, replication and recombination. In eukaryote studies almost all current work is directed towards the question of the control of selective transcription. By analogy with the known control sequences in bacterial genes this has led to the search for common elements in the 5′ sequences flanking the gene. But presentations on *Xenopus* 5S RNA genes, sea urchin histone genes, human globin genes and chromatin reconstruction experiments suggest that the control signals need not be in the adjacent 5′ sequence and the 5′ signals that do exist may not be obvious at the DNA sequence level. Bogenhagen (Carnegie Institution, Baltimore), working with D. Brown, has been asking what sequences of a cloned 5S RNA gene are important for transcription, using the ability of appropriate extracts of *Xenopus* oocytes to obtain precise transcriptional initiation and termination of a cloned 5S RNA gene.

The experiments show that all of the 5′ flanking sequence and up to 50 base pairs into the gene may be replaced without substantially affecting initiation of transcription. It follows that the 5′ flanking sequences and indeed the first 50 bases of the gene itself do not influence the site of initiation. By progressive deletion from the 3′ end of the gene and measuring initiation in the presence of the chain-terminating analogue cordycepin, an intragenic control sequence of 30 base pairs was found. The next step was to insert these 30 base pairs only into a bacterial plasmid and show that the *Xenopus* RNA polymerase III initiated transcription in plasmid DNA 50 base pairs 'upstream' of this sequence in either orientation. So what is this 'brown box' sequence doing? There are a number of possible explanations but Bogenhagen pointed out that 50 base pairs is 170 Å long and RNA polymerase III is big enough to contact both the intragenic promoter and the 5′ initiation point. How many genes have 'brown boxes'? The VA RNAs of adenovirus are transcribed by RNA polymerase III, have a known sequence and are amenable to genetic analysis; these must also be good candidates for intragenic control sequences.

Surrogate genetics

Eukaryote genes coding for proteins are transcribed by RNA polymerase II which has only recently been persuaded to initiate faithfully *in vitro* (Weil *et al. Cell*, **18**, 469; 1979). M. Birnstiel (University of Zurich) reported on experiments where genetically manipulated sea urchin histone genes are functionally assessed *in vivo* by microinjection into the nucleus of a *Xenopus* oocyte. The histone genes occur in repeating clusters each containing H4, H2b, H3, H2a and H1 in this order. In the oocyte only H2a and H2b proteins are unequivocally detected when a plasmid containing all the histone genes is injected. Comparison of the noncoding sequences at the 5′ and 3′ ends of the H2a repeated genes shows conserved regions. The implication is that these regions have a conserved function and the Zurich group is testing whether this is transcriptional control by deleting these sequences and studying RNA transcripts in the microinjection assay. An attractive feature of the system is that H2a may be manipulated while leaving the H2b sequence upstream as an internal control presumably not subject to polar effects. The Zurich group does observe alterations in the amount and size of the H2a transcripts when noncoding 5′ sequences are altered *in vitro* although the possibility that RNA processing may be responsible cannot be excluded.

Both the Baltimore and Zurich groups are approaching the question of what is a transcriptional unit using the same logic, of isolation, manipulation and functional assay. R. Flavell (National Institute for Medical Research, London) in contrast, reviewed the descriptive studies of the deletions that give rise to thalassemias in man. He pointed out that deletions of the delta and beta globin gene sequences alter the expression of the fetal globin genes in adult life even though these fetal genes lie more than 10 kilobases to the 5′ side of the deleted sequences. There is some tentative evidence that these long range interactions may be a *cis* acting effect. We can conclude that in these three eukaryotic systems our traditional view of what constitutes a gene will be changed. In the case of the *Xenopus* 5S gene there is no doubt that an important new means of exerting genetic control has been described.

Chromatin structures

A. Klug (MRC Laboratory of Molecular Biology, Cambridge) reviewed the impressive evidence accumulating for the solenoid model of nucleosome superstructure from neutron scattering, electron microscopy and, surely definitively, from the X-ray patterns of intact nucleosome crystals. The concern of the Cambridge group has been to describe the higher order structure of chromatin. P. Chambon (University of Stransbourg) on the other hand addressed the question of whether the structural arrangements of DNA in chromatin affect gene expression. The DNase I hypersensitivity of active genes first described by Weintraub now seems to be generally true but as Chambon pointed out it is not known whether this is the cause or consequence of gene activity. Nor is it clear what constitutes a 'domain' of hypersensitivity. In the SV40 minichromosome Varshavsky has shown that a region around the origin of replication is highly susceptible to enzymatic digestion because it is not packaged into nucleosomes. Chambon's group has now shown that this open structure can be obtained on reconstituting SV40 chromatin. The importance of this result is that it shows that the DNA sequence is itself responsible for the 'open' arrangement of nucleosomes around the origin. So in this case at least we can conclude that hypersensitivity to nuclease digestion precedes the expression of the viral genome on infection.

RNA processing

J. Abelson (La Jolla) has made progress in characterising the yeast tRNA splicing activity he has described. The possibility that this 'tRNA spligase' may also be an 'mRNA spligase' was raised by the extracurricular reports of intervening sequences in the yeast actin genes both from Abelson's laboratory and from Gallwitz (Marburg). Irrespective of whether the same enzyme is involved in both tRNA and mRNA splicing, the ease and precision of yeast transformation means that this will be an important organism in the study of the biochemistry and genetics of splicing. H. Robertson (Rockefeller University) reported on work showing that the influenza virion RNA polymerase activity captures the capped 5′ end of host mRNA not by splicing but by using the cleaved 5′ oligonucleotides as a primer for transcription.

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*The EMBO Symposium on Nucleic Acid — Protein Interactions was held in Heidelberg on 11-15 October, 1979.

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Real genetics

Real *Drosophila* genetics has shown that the eye colour mutant white/crimson (W^C) can frequently move its location. W. Gehring (Biozentrum, Basel) in looking for cloned heat shock genes has found a 'copia-like' element (P6F14) that localises to sites predicted by genetic mapping to contain the unstable W^C gene. The so-called 'copia' elements are a family of sequences present at around 30 sites on the chromosomes and which apparently can insert into new sites analogously to the better-known bacterial transposons. The significance of this work is that the dispersed and unstable copia DNA element has picked up a phenotype — in this case the white/crimson. Gehring indicated that in certain stocks the W^C gene may be left in a new location while the unstable element acquires a new phenotype.

M. Ptashne (Harvard University) has used genetic manipulation to mix up the control sequences and proteins of the *lac* genes and the lambda repressor and *cro* gene products. Coupled with DNase protection (footprinting) experiments using the lambda repressor and *cro* gene product he derives an elegant explanation of the role of the three tandem binding sites at the O_R region. There are at least four lessons from this work: (1) 'completely' different proteins can bind to the same DNA sequence; (2) the same protein can be both a positive and negative transcriptional regulator; (3) cooperative interaction between proteins determines transcriptional control and prophage induction is achieved by *rec*A mediated abolition of cooperativity; (4) all temperate phages have three binding sites governing the lytic/lysogenic choice; this arrangement probably prevents frequent mutation to virulence. There is no doubt that the O_R system is the best defined example of the control of transcription. In this case the difference of one base pair in the size of the spacer between the sites is thought to determine the direction of transcription. A. Landy (Brown University) has also used the footprinting technique and genetic engineering to show that the phage λ integration (*int*) gene product interacts with 240 base pairs spanning the core *att* site (at which λ integrates). This promises to explain the mechanism of at least one kind of site-specific recombination. It was left to F. Stahl (University of Oregon, Eugene) in summarising the meeting to make sure that participants obtained a working knowledge of the role of bacterial *chi* sequences in generalised recombination. The implication is that soon bacterial recombination and transcription will be fully explained in terms of known proteins interacting with characterised DNA sequences, leaving the many mysteries of eukaryotes to keep us amused.

Fe II emission lines in astronomy

from Correspondents

IN October the European Space Agency (ESA) sponsored a workshop on the subject of Fe II emission lines in stars and galaxies held at its Villafranca Satellite Tracking Station near Madrid, Spain — also known as VILSPA. It is from this station that ESA carries out its part in the operations of the International Ultraviolet Explorer (IUE) satellite (see *Nature* **275**, 5 October; 1979) which can observe the resonance lines of ionised iron in astronomical objects without high redshifts. Before IUE, many objects showing optical emission lines of this ion were known and with the early IUE data came the chance to check theoretical models for the emission from this complex ion by studying the key resonance lines.

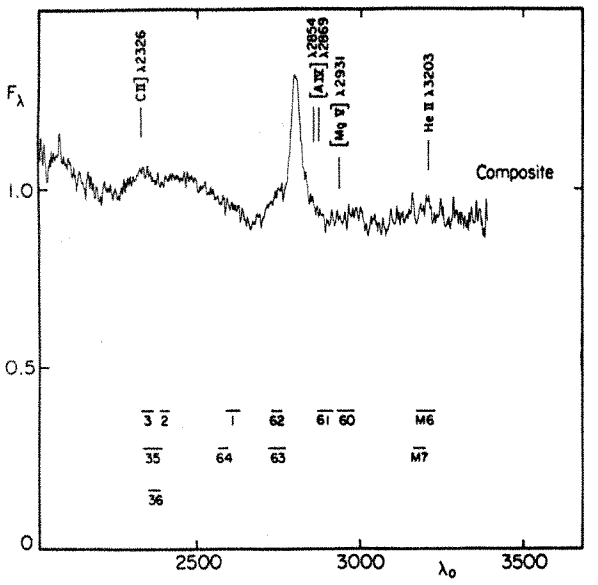
Emission lines of ionised iron are widespread in astronomical objects and come from the Sun, all cool stars, some hot stars (in particular those with emission lines, those that are superluminous, the so-called symbiotic stars and binaries), eruptive objects like novae, supernovae, occasional very dense nebulae and extragalactic objects such as quasars and the related Type 1 Seyfert galaxies. In the case of galactic objects these emissions are known to arise in the cool parts of extended envelopes around stars. Typical electron densities and temperature run in the ranges 10^5–10^{11} cm^{-3} and 8,000–10,000 K. Where the densities are less than about 10^8 cm^{-3}, forbidden [FeII] emission is expected but J.-P. Swings (Liege) stressed that deducing the density from the ratio of forbidden-to-permitted lines may not always be justified since the lines' profiles may differ sharply between forbidden and permitted lines. Generally the lines that are seen in the optical and ultraviolet emanate from the odd levels around 5 eV, however M. Penston (VILSPA) and M. Snijders (University College London) reported the presence of several strong ultraviolet lines from even 10 eV levels in the slow nova RR Tel and in Nova Cygni 1978 respectively. Both Swings and R. Viotti (Frascati) noted the complex behaviour of the lines, with the relative importance of absorption and emission varying greatly between multiplets and between lines in the same multiplet. M. Friedjung (Paris) reported on the success of curve-of-growth type methods of analysing the Fe II emission spectra of several sources. It was generally agreed that the early investigations of the Fe II emission spectrum in galactic objects had lacked the quantitative dimension now available in the ultraviolet and regularly provided in the optical by modern observing methods: some new spectrophotometry was thought to be important.

Among extragalactic objects Fe II emission is seen in quasars and the related Seyfert type 1 galaxies. The same multiplets are strong in galactic sources and are identified in the optical region while [Fe II] may be marginally present in one case, IZW1, which has very strong Fe II. There is a great variation in the strength of Fe II relative to other lines or to the continuum. Of the Fe II multiplets, 42 may vary in strength with respect to the others from object to object. One of the most exciting results of the workshop was the convergence of many sets of data finding the same low contrast emission features between 2,000 and 3,000 Å. Both ground-based data on quasars with moderate redshifts by M. Phillips (Cerro Tolodo) and H. Netzer (Tel Aviv) and IUE data on low redshift Seyfert galaxies by Snijders and J. Clavel (VILSPA) agreed on the widespread existence of the features illustrated in the Figure. There was however some discussion of the identification of these features: although Fe II may be responsible, other explanations are not yet completely excluded and more work on this point will be done. Generally the intensity of these ultraviolet Fe II features is about equal to that of the optical Fe II lines in spite of factor 100-1000 differences in branching ratios. In addition the IUE observations of Seyfert galaxies with strong optical Fe II may show the presence of some features which could be ultraviolet multiplets in the wavelength range 1600-2000 Å (not previously seen in quasars) and a possibly anomalously strong Si III] to C III] ratio, which would indicate a high density.

Theoretical discussion centred on the Fe II lines in quasars and active galaxies and turned on the energetics. It was agreed that energy can be deposited in the neutral hydrogen, ionised iron region by photoionisation or collisional ionisation. Netzer presented models using a 'mean escape probability' method to handle the radiation transfer which showed that photoionisation by the X-ray continua of active objects could deposit adequate energy in these regions to create the observed Fe II and Mg II lines. Similar calculations by S. Collin-Souffrin and M. Joly (Paris) with a more detailed treatment of the radiation transfer also considered the effects of collisional ionisation. In spite of the difference in fundamental energy source, there was a general similarity in the emergent spectrum which can fit the observed intensity ratios of Mg II to Fe II and of Fe II (ultraviolet) to Fe II (optical). It was also agreed that the excitation mechanism was collisional and not from the resonance fluorescence process. The main argument against the latter is that observations at the Lyman limit in high redshift quasars suggest only a covering

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An example of the data presented at the workshop which converged in showing substantial evidence for repeatable low-contrast features in the spectra of quasars and Seyfert galaxies between emitted wavelengths of 2000 and 3000 Å. (after Wills, Netzer, Uomoto and Wills)

factor of 5% or less by the emission line region: this proportion of the continuum absorbed by the Fe II ultraviolet resonance lines would be insufficient to explain the optical emissions. It was felt that the models, while capable of predicting the total intensity of the Fe II lines or the ratio of optical to ultraviolet lines, could not yet be used to predict ratios of individual multiplets because of the inaccuracy of the atomic physics data. Thus for example problems like the variable ratio of multiplet 42 (sextet) to other optical (quartet) multiplets, although explained qualitatively by C. Jordan (Oxford), could not yet be tackled quantitatively. As for the resolution of the fundamental choice of photoionisation and collisional ionisation of the Fe II region, it was agreed that the hydrogen lines offered the best diagnostic. There was as a result some discussion led by G. Ferland (Cambridge) of the anomaly seen in quasars and active galaxies where the hydrogen Lyman line is too weak relative to the Balmer lines by a factor of ten compared with radiative recombination.

Because of the interlocking between ultraviolet and optical region transitions, it emerged that the line formation process is complex and that atomic data for many transitions are required. The physical processes of interest include collisional excitation, photo-excitation by strong ultraviolet lines, line self-absorption and recombination. Until now oscillator strengths have been calculated using simple approximations (for example, using the Thomas-Fermi potential) and with a limited number of configurations. Comparison with the available experimental data indicate that the calculated values are reliable only for the stronger lines if l-s coupling is valid; however spin-forbidden and two electron transitions are particularly important for Fe II. There was therefore an urgent need for more sophisticated multi-configuration calculations also allowing for spin-orbit interaction and for further measurements particularly in the ultraviolet region. Collisional excitation cross sections are also required and the commonly used simple approximations are unlikely to be appropriate. Although for high precision the close-coupling approximation will be needed, M. Seaton (UCL) pointed out that the distorted-wave method will give results of sufficient accuracy to be useful, given the state of the observations, and analysis of extragalactic spectra demands only somewhat crude atomic data. A start on this work has been made by H. Nussbaumer (Zurich) and P. Storey (UCL), stimulated by the workshop and new extragalactic results. They used the close-coupling method and a two-configuration basis to obtain collision strengths among the lowest four terms. The workshop also noted that processes involved in the ionisation and recombination of Fe II merit closer examination. Lastly a good knowledge of the energy level structure is often taken for granted. Yet astrophysicists are entirely dependent on fundamental laboratory spectroscopy, such as the excellent work at Lund reported to the workshop by S. Johansson, to begin their studies. It was agreed that such fundamental work ought to continue because of its immediate applications in explaining the many remaining unidentified transitions in the Sun and stars and in the theoretical modelling of the atoms. □

Reindeer and caribou

from Eigil Reimers and David R. Klein

REINDEER and caribou (all species of *Rangifer tarandus*) occur wild and domesticated in the northern circumpolar countries, and have been introduced into the Southern Hemisphere on South Georgia. Reindeer and caribou live in a variety of different environments and the second international reindeer/caribou symposium* provided an opportunity for comparing the different characteristics of these environments and the problems they produce. The endemic reindeer of the Svalbard Archipelago (Spitzbergen) for example, have no natural predators to contend with, are free from harrassing insects and are not hunted; in Greenland predators are absent; in Scandinavia and North America reindeer and caribou are subject in varying degrees to all three of these factors which are important limiting factors on population.

The wide population fluctuations that have occurred in wild reindeer and caribou populations and specific population regulation mechanisms can be considered from two main viewpoints. One considers hunting and predation as the main cause of population fluctuation, with overharvest by the two leading to population decline. Hunters and predators would as a consequence either be reduced in numbers or be forced to explore other sources of food, allowing the population to increase again.

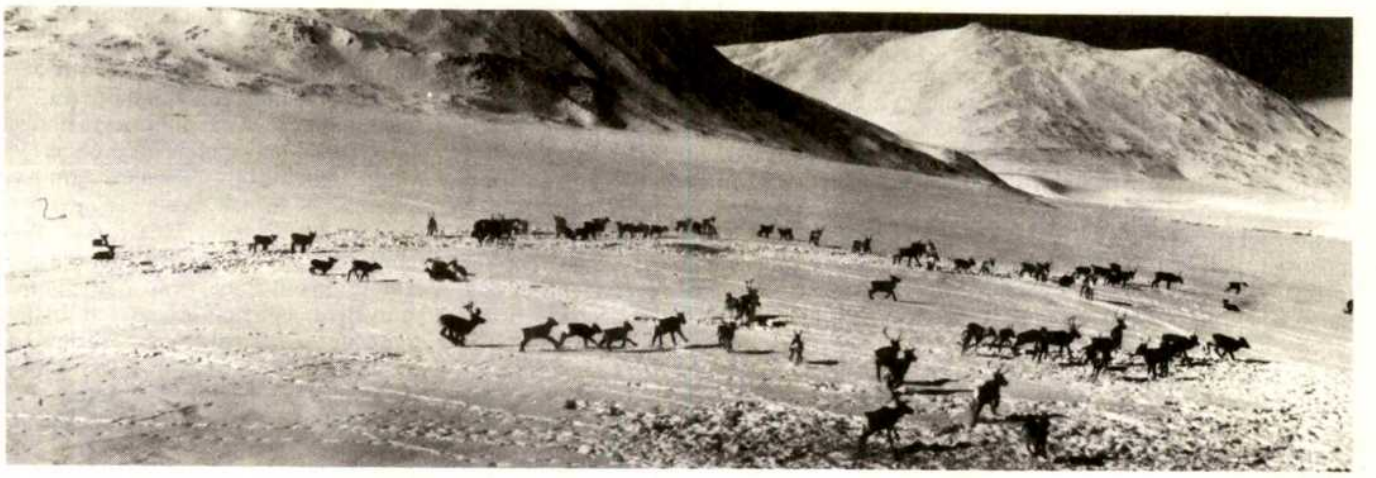
The other viewpoint focuses on the range resources and the 'predation' on them by the reindeer and caribou. As the population increases, the range plant biomass will decrease, leading to poor physical condition of the animals, higher mortality and lower reproduction rates, eventually leading to population decline. With a small population the vegetation will recover and the population increase again.

In Svalbard, Scandinavia and Greenland the latter viewpoint is probably the more correct. For example, the recent decline in wild reindeer in Greenland is certainly associated with poor nutrition, caused by overgrazed ranges combined with a heavy load of parasites and disease. The situation there has been worsened through the introduction of parasitic skin warble and nasal bot flies in the 1950s when domestic reindeer were introduced.

Reindeer can use a wide variety of food species. Whereas lichens are the primary winter food base for reindeer and caribou on continental ranges, in the High Arctic mosses are important, as well as vascular plants which are of minimal importance further south. To understand animal range relationships properly considerable attention is now being given to assessing activity budgets. These, and especially feeding activity, reflect the balance between food intake and energy expenditure and therefore indicate the condition of the animals as well as that of the range. In this context Svalbard reindeer

*The 2nd International Reindeer/Caribou Symposium was held in Røros, Norway on 17-21 September, 1979. The proceedings will be published in 1980 by the Norwegian organising committee (Second International Reindeer/Caribou Symposium, Institute of Zoophysiology, University of Oslo, PO Box 1051, Blindern, Oslo 3, Norway). The proceedings will include a special section on the present status of *Rangifer* populations throughout the world. The third symposium is scheduled for Finland in 1982.

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Caribou in the Canadian North

are receiving special attention because of apparent physiological adaptations to their especially extreme environment so far north. They accumulate large quantities of fat, they feed heavily on mosses which are avoided elsewhere and conserve energy through reduced activity especially in summer. In spite of the lower plant biomass and annual net primary production in Svalbard compared with areas further south, Svalbard reindeer have a greater population density and faster growth rates. Techniques now in use in studies of reindeer ecology run from remote satellite and aircraft sensing to map range and snowcover as well as to assess range quality, to radionucleotide tracer methods used to establish body nitrogen turnover and to estimate energy flow through the lichen-caribou-wolf chain throughout the year. Assuming that the wolves take a constant number of caribou throughout the year, the wolf harvest of caribou was estimated to be 30 ± 5 animals per wolf each year.

Serological techniques for assessing genetic differences and similarities among reindeer and caribou are contributing to the clarification of relationships between the circumpolar racial groups. The Svalbard reindeer are quite unrelated to the reindeer of the Scandinavian peninsula and comparisons with the Peary caribou of the Canadian Arctic have been initiated as an outcome of the symposium.

Man-made obstructions and harrassments such as aircraft, pipelines, highways, railroads and powerlines have pronounced effects on the movement, feeding activity and hence energy budgets of reindeer and caribou herds, and the disruption involved in building and servicing oil pipelines for example, with the associated traffic and helicopters, leads to alarm and avoidance reactions especially amongst females accompanied by young. Another unexpected and undesirable side-effect of man's activities has been seen in Sweden and Finland where the fertilisers used in the forestry industry seem to inhibit the winter foraging for lichens through the snow in these areas, apparently as a result of odours released by the ammonium nitrate and urea fertilisers. □

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The neutron half-life and cosmology

from R.J. Tayler

THE recent announcement by Bondarenko *et al.* (*Sov. Phys. JETP Lett.* **38**, 303; 1978) of a new measurement of the neutron half-life giving a value of 10.13 minutes instead of 10.61 minutes, serves as a reminder that this value is of fundamental cosmological importance. A further measurement of the half-life has recently been made at Harwell and a preliminary analysis of the results suggests that the value obtained will also be less than 10.6 minutes (J. Byrne, private communication).

Since the discovery of the microwave background radiation in 1965 there has been a general hope that the hot big bang cosmological theory, originally proposed by Gamow, provides a good first approximation to a description of the evolution of the Universe. One of the key predictions of this theory is the chemical composition of the Universe after the first few minutes. This should be about three parts hydrogen to one part helium by mass, with a very small admixture of deuterium, ^{3}He and ^{7}Li. The precise division between hydrogen and helium and the other light isotopes depends on how rapidly the Universe expands in its early stages and on the relative number of protons and neutrons at the time that nuclear reactions converting hydrogen into helium become effective. The simplest version of the big bang theory has one free parameter which can be related to the present mean density of the Universe (ϱ). The lower the value of ϱ_0, the lower also was the helium production in the early Universe. There is an uncertainty of about two orders of magnitude in ϱ_0 and this corresponds to a helium production of between 0.23 and 0.27 by mass.

The rate of expansion of the Universe is increased if there are more stable elementary particles than is at present believed. In the very early Universe all particles and their antiparticles are expected to be present in equilibrium with the radiation field and stable massless or low mass particles survive through the period of nucleosynthesis and influence the expansion of the Universe. If, as seems very likely, a third neutrino, the tau neutrino, exists, the predicted helium production is increased to the approximate range 0.25 to 0.29. At present there is no unanimity amongst elementary particle physicists about the number of leptons and associated neutrinos, but if there are more than three sets a further increase in helium production is implied.

The half-life of the neutron enters into the problem because it affects all the reactions which convert neutrons into protons. If the half-life is reduced, neutrons are more readily converted into protons and the helium production is reduced. When Gamow first proposed his theory, the half-life was believed to be 14 minutes. By 1965, when the first very serious comparisons between theory and observation were made, the value 11.7 minutes was used and the probable helium production fell in the range 0.25 to 0.29. This was reduced to the 0.23 to 0.27 quoted above, when the accepted value of the half life became 10.6 minutes. A further reduction to 10.1 minutes would give the values 0.22 to 0.26, or 0.24 to 0.28 if the tau neutrino is included.

When the predictions of the theory are compared with all relevant observations, including those of element abundances and those bearing on the value of ϱ_0, it seems just possible that the simplest version of the theory is compatible with observation. Most observed helium abundances are greater than 0.23, although considerably lower values have been observed in the solar wind. It is usually believed that this results from selective loss of elements by the Sun, although Isaak (*Nature*, in the press) has recently claimed that observed solar oscillations and a low neutrino flux demand a low helium content. If we dis-

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regard this rather controversial point, the tightest observational constraint on the theory is given by the deuterium abundance, which although very small depends very strongly on the rate of expansion of the Universe.

This agreement between the simplest theory and observation would probably be removed if even a fourth or more species of neutrino were discovered; *inter alia* the predicted helium abundance would be too large. There are more complicated versions of the theory which involve the Universe's being filled with degenerate neutrinos or antineutrinos, which might fit the observations, but these seem rather unattractive. A reduction in the neutron half-life of the amount found by Bondarenko *et al.* would make a small correction in the opposite direction. However, the main significance of the result for cosmologists is to remind us that the predictions of the hot big bang theory are not secure until the neutron half-life is really well known. A definite result that it could not be less than 9.5 minutes (say) would be extremely helpful. □

Amorphous semiconductors

from S.R. Elliott

THE dominant theme at a recent conference on amorphous and liquid semiconductors*, was the hydrogenated amorphous silicon (a-Si:H) system. This is perhaps not too surprising in view of its possibilities as a device material, for example in solar cells (see *News and Views* **275**, 93; 1978; **277**, 85; 1979). Many of the papers on a-Si:H dealt with aspects of material preparation (for example glow-discharge decomposition of SiH_4 or sputtering in Ar/H_2 atmospheres) or characterisation, such as the measurement of H content by thermal exodiffusion or infrared spectroscopy, and investigations of gap-state density by various field-effect or capacitance-voltage measurements. Few structural studies were reported, although R. Mosseri *et al.* (CNRS, Meudon) compared X-ray diffraction data on a-Si and a-Si:H, and D. Weaire *et al.* (Heriot-Watt University) discussed a hand-built 'ball-and-stick' structural model for a-Si:H containing about 20 at.%H. An entire session was devoted to photovoltaics and solar cell devices, discussed by workers from RCA, Exxon, Dundee and Osaka, and I. Shimizu *et al.* (Tokyo Institute of Technology) described how a-Si:H films could also act successfully as electrophotographic sensors in a manner similar to another amorphous material, selenium, used in copying machines.

A. Madan and S.R. Ovshinsky (Energy Conversion Devices) reported on fluorinated silicon, a-Si:F:H, (made by the glow-discharge decomposition of SiF_4/H_2 mixtures) which, they claimed, has a lower density of gap states than solely hydrogenated material and which therefore should lend itself to more efficient doping (see also *Nature* **276**, 482; 1978). Indeed, for material containing about 5 at. % F, doping with AsH_3 or PH_3 in the gas phase produced material having, at the highest doping levels, conductivities some three orders of magnitude higher than comparable hydrogenated material, although p-type doping with B_2H_6 was found to be comparable in both cases. With a band-gap some 10% larger (rather more suited to absorption of the solar spectrum), a-Si:F:H looks as if it might have considerable device potential. J. Knights (Xerox) presented an interesting paper on growth and morphology of a-Si:H, in which strong evidence from nuclear magnetic resonance, electron microscopy and other techniques was presented for the inhomogeneous incorporation of H. Previously, it had generally been believed that H was uniformly distributed throughout the bulk; Knights, however, showed that high H-containing samples were formed from clusters about 100 Å in diameter, with the H concentrated at the cluster boundaries in the form of SiH_2 configurations. Lower H-containing samples did not show this island morphology, but appeared homogeneous. Plasma chemistry seems to play a major part in the deposition process, as might be expected.

Chalcogenide glasses, the other main area of amorphous semiconductor research, also received much attention. The major breakthrough in this field in the past two or three years has been the realisation that intrinsic structural defects have a major role in determining many electronic properties of these materials. These point defects — dangling bonds — are believed to undergo reconstructions in which electron pairing at these sites occurs. Thus, considering a-Se as an example (in which normally bonded atoms are 2-fold coordinated, forming chains), a simple dangling bond is 1-fold coordinated and contains one electron in the orbital formerly used in bonding. Reconstruction occurs by the transfer of an electron between two such defects: addition of the electron to a centre results in a negatively charged 1-fold coordinated centre: removal of an electron results in an empty orbital capable of forming a dative bond with the full lone-pair orbital on a neighbouring Se chain, thereby forming a positively charged 3-fold coordinated centre. J. Joannopoulos (Massachusetts Institute of Technology) described some of the first self-consistent calculations on such defect configurations in an attempt to obtain total energies. However, it seems that, at least at present, the inability to model the atomic relaxations in a realistic manner leads to estimates accurate only perhaps to 0.5 eV, unfortunately not precise enough to determine the relative stability of various configurations.

B. Golding (Bell Laboratories) described some fascinating experiments probing low-lying excitations — so-called two-level systems — which seem to exist in chalcogenide glasses. These had been thought to be due to the tunnelling of atomic groups between two nearly equivalent configurations, and they dominate the thermal properties below 1 K. Stimulated emission (in the form of ultrasonic or electromagnetic echo radiation) from these two-level systems can be obtained, and it was shown that such electrical echoes could be altered in magnitude by annealing or by irradiation with light having the energy of the band-gap (or half-band-gap) of the material. The importance of this result lies in the fact that these changes are precisely in the same sense as those observed in photo-luminescence or electron spin resonance under the same conditions, properties which are believed to be due to the structural defects mentioned earlier. This is the first direct indication that a connection may exist between two-level systems and structural defects in chalcogenide glasses, an intriguing possibility.

G. Pfister (Xerox) reported on the incorporation of additives (such as transition and alkali metals) in chalcogenide glasses and the effect on properties such as drift mobility and photoluminescence. The discovery that, although the former is dramatically affected, the latter is relatively unaffected by doping means that the simple model for defects in chalcogenide glasses needs to be modified. On a more technological note, a novel method of high resolution image replication using amorphous Ge–Se films was described by K. Chopra *et al.* (Indian Institute of Technology, Delhi). This technique relies on the enormous thickness changes (about 12%) that occur when obliquely deposited films are illuminated with band-gap light; this method has the advantage of being conducted under dry conditions, unlike conventional photolithography.

Finally, the prize for the most unusual and esoteric method of glass production must surely go to a joint Czech and Russian group (L. Stourac *et al.* Czech Academy of Sciences, Prague & Institute of Space Research, Moscow). The experimental environment they used was the weightless condition aboard the Salyut 6 spacecraft, and glass formation and the influence of zero gravity on the structure and opto-electronic properties of Ge-Sb-S glasses were studied. I fear that it will be a little time yet before British researchers have an opportunity to conduct similar experiments! □

*The Eighth International Conference on Amorphous and Liquid Semiconductors was held on 27-31 August, at Harvard University. The Proceedings are being published as a separate volume of the *Journal of Non-crystalline Solids*.

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articles

Flux variations of QSO 0957+561 A, B and image splitting by stars near the light path

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If the double QSO 0957+561 A, B is the result of gravitational lens actions by a massive galaxy, stars in its outer parts and close to the light paths may cause significant flux changes in one year. One star can split a QSO image into two to four images with angular separations of $\sim 10^{-5}$ *arc s.*

RECENT observations[1-3] of the double QSO, 0957+561 A, B (redshift $z = 1.405$, angular separation $\alpha = 5.7$ arc s, mag ≈ 17) strongly indicate that a large and compact mass (galaxy) lies close to the line of sight between Earth and the QSO. The massive galaxy thereby acts as a gravitational lens. The theory for the case of a point mass (or a compact spherically symmetrical mass distribution) has been presented elsewhere[4-8,14]. The most convincing proof of a lens effect would be the demonstration of a time delay (of months or years) between flux fluctuations in the two QSOs, which would occur by intrinsic fluctuations in the QSO, as the light travel time is different along the two light paths[5,6,9,10,14].

For source diameter <1 lt yr we show here that flux fluctuations (time scale of months and more) can also be caused by stars, in the outer parts of the deflecting galaxy, moving close to one of the light paths. Such flux fluctuations in the two images would certainly be uncorrelated and show no time delay effect. The absence of a time delay effect should therefore not automatically be taken as disproving the lens effect. The observed light curves would probably show variations due to intrinsic variations in the QSO as well as the influence from the above-mentioned stars. The presence of a time-delayed component in the observed fluctuations would, however, not be difficult to demonstrate.

To simplify the discussion, we consider here the effect of only one star at a time, which is sufficient to demonstrate the importance of stars close to the light path. We shall see that an interesting aspect of the problem is the splitting of each image into a close group of images with angular separation of $\sim 10^{-5}$ arc s. Even one star alone can cause a QSO-image to split into two to four images. Of particular interest is the transition (due to proper motions) from four to two images (or the opposite). Before such a transition occurs, two of the four images are seen to move towards each other, get brighter, and then merge and disappear. The time scale for the disappearance of the two images will roughly be the time for the QSO to move transversely its own diameter D and can be estimated to be about $600 D\, c^{-1}$ in the present case. For D values of <0.1 lt yr, flux variations of 50% and more can occur and even for D values of ~0.5 lt yr a variation of 5% during 30 yr is possible. For such large D values, however, the different images discussed above will usually merge into one image. From the light curves of the double QSO it should be possible to get information on the size and structure of the QSO as well as on the mass distribution in the outer parts of the deflecting galaxy.

Gravitational lens geometry

The geometry of our problem is indicated in Fig. 1. The heavy solid line represents a light ray coming from Q which is deflected (by the galaxy (G) and the star (S)) when crossing the deflector plane at the point (ξ, η) and which finally crosses the observer's plane at the point (x, y). The affine parameters, L and λ, are convenient distance parameters for gravitational lens problems at cosmological distances[11,12]. One might at first think that the Einstein-deflection arising from the nearest star can be neglected since it is only $\sim 10^{-6}$ times the galaxy deflection

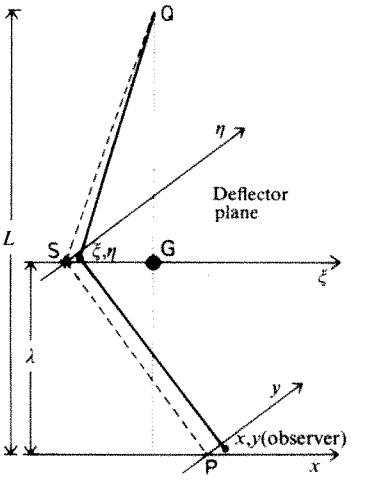


Fig. 1 Light source Q with the affine parameter L (ref. 11) large deflector G (galaxy) with the affine parameter λ and small deflecting mass S (star). The deflector plane (ξ, η) and the observer's plane (x, y) are normal to the line QG. For convenience we chose the origin of the (ξ, η) plane to be at S and the x axis to be parallel to the ξ-axis. The origin P in the (x, y) plane is obtained by considering a light ray QSP for which only the galactic deflection is taken into account. The solid line from Q through the points (ξ, η) and (x, y) represents the path a photon would take when the deflections from G and S are taken into account.

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($M_G/M_S \approx 10^{12}$). For the observed fluxes in the observer's plane, however, it is not the values of the deflection angles which count, but rather their derivatives with respect to the deflector plane coordinates, and these derivatives turn out to be of the same order of magnitude.

Basic equations

A suitable form for the transformation of the (ξ, η) plane into the (x, y) plane for the light rays from a point source is the following:

$$x = \xi + \gamma\xi - \xi/(\xi^2 + \eta^2) \quad (1)$$

$$y = \eta - \gamma\eta - \eta/(\xi^2 + \eta^2) \quad (2)$$

The galaxy has here been treated as being symmetrical about QG and with negligible mass outside S. The deflection due to the galaxy is a function of ξ and η and is taken to be the deflection in $\xi = \eta = 0$ plus a term linear in ξ and η. Such a power expansion is a very good approximation since we shall only be interested in light rays which pass S at distances ≤ 1 lt yr. The unit of distance

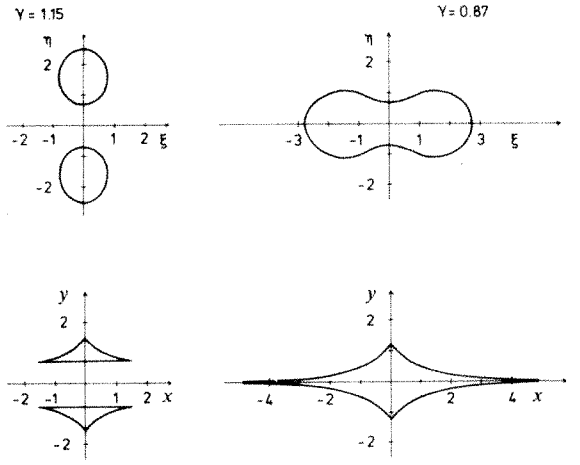


Fig. 2 Critical curves in the deflector plane (ξ, η) and in the observer's plane (x, y) for the cases $\gamma = 0.87$ and $\gamma = 1.15$.

in the (ξ, η) plane has been taken to be the radius r_0 of the luminous ring which would have been seen only if the star had been acting as a lens with the observer directly behind the star. The value of r_0 is[4,12] (normalised unit),

$$r_0 = \frac{2}{c}\left(GM_S \frac{\lambda(L-\lambda)}{L}\right)^{1/2} \quad (3)$$

For $M_S \approx 1\ M_\odot$ and for cosmological distances the value of r_0 is of the order of 0.1 lt yr. Finally, the normalised unit in the (x, y) plane is $r_0 L/(L-\lambda)$. An interesting feature of equations (1) and (2) is that there is only one free parameter γ and that the γ-terms arise from the galactic deflection.

If we neglect the effects of the deflecting star, we can drop the third term on the right-hand side in equations (1) and (2):

$$x = \xi + \gamma\xi \quad (1a)$$

$$y = \eta - \gamma\eta \quad (2a)$$

We denote the flux which would have been observed without any lens effect by F_0 and the corresponding flux if only the galactic deflection is taken into account by F_0'. It is then easily seen from geometrical optics that

$$F_0'/F_0 = |J|^{-1} \equiv \left|\frac{\partial(x, y)}{\partial(\xi, \eta)}\right|^{-1} = |1 - \gamma^2|^{-1} \quad (4)$$

where J is the jacobian determinant of the transformation. The flux ratio of the two observed images A and B of the QSO is $F_A/F_B \approx 4/3$ from the available radio observations[2]. Using the conventional lens effect analysis[4] (galaxy only) one finds furthermore

$$F_A - F_B = F_0 \quad (5)$$

so that

$$F_A = 4F_0,\ F_B = 3F_0 \quad (6)$$

We then get an approximate estimate of γ by inserting F_A and F_B instead of F_0' in equation (4):

$$\gamma_A \approx (1 - F_0/F_A)^{1/2} \approx 0.87 \quad (7)$$

$$\gamma_B \approx (1 + F_0/F_B)^{1/2} \approx 1.15 \quad (8)$$

Equation (4) does in fact allow four solutions of γ for a given flux ratio. It is, however, easy to see that γ must be positive when $M_G > 0$. We also know that the bundle corresponding to the brightest image A has not been through a caustic between source and observer whereas 'bundle B' has been through a caustic. From this it follows that $\gamma_A < 1$ and that $\gamma_B > 1$ and the values given in equations (7) and (8) then follow as the only possible values of γ_A and γ_B.

Critical curves and number of images

An interesting property of equations (1) and (2) is that the inversion of these equations leads to fourth order equations in ξ and η and that one can have two to four solutions for a given x, y. This means that up to four different light rays passing close to S can reach the same point in the observer's plane, so that the observer will see a group of 2–4 images (angular separation $\sim 10^{-5}$ arc s).

To find out how the number of solutions depends on the observer's position (x, y), the location of the points for which the jacobian of x and y in equations (1) and (2) is zero turns out to be very important:

$$J(\xi, \eta) = \frac{\partial(x, y)}{\partial(\xi, \eta)} = 0 \quad (9)$$

Equation (9) defines the 'critical curves' in the (ξ, η) plane. The transformation of these curves into the observer's plane by equations (1) and (2) then defines the critical curves in the (x, y)

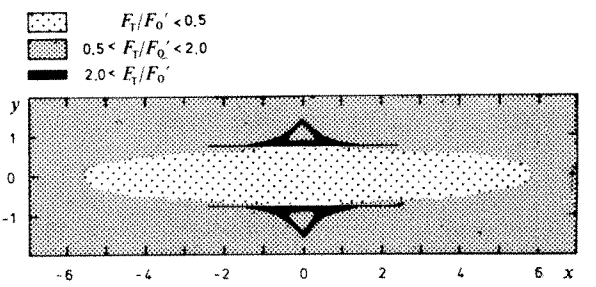


Fig. 3 The observer plane, for the case $\gamma = 1.15$, divided into a high flux region, an intermediate flux region and a low flux region. F_0' is the flux which would have been observed, if only the galactic deflection is taken into account (the star is neglected).

plane. The critical curves in the (ξ, η) plane are found to be Cassini ovals and they are plotted for the cases $\gamma = 0.87$ and $\gamma = 1.15$ in Fig. 2 together with the corresponding critical curves in the (x, y) plane. These two cases also demonstrate the two types of critical curves one can obtain. For $\gamma < 1$ one closed curve is always found in each plane; for $\gamma > 1$, however, there are always two closed curves.

By using topological arguments one can show that the number of images of a point source seen by an observer can only change when he crosses a critical line, and that the number of images then changes by two. This is also in perfect agreement with our numerical experience; we always find that the observer sees two images when he is outside the critical curve(s) and four solutions when he is inside one of the critical curves.

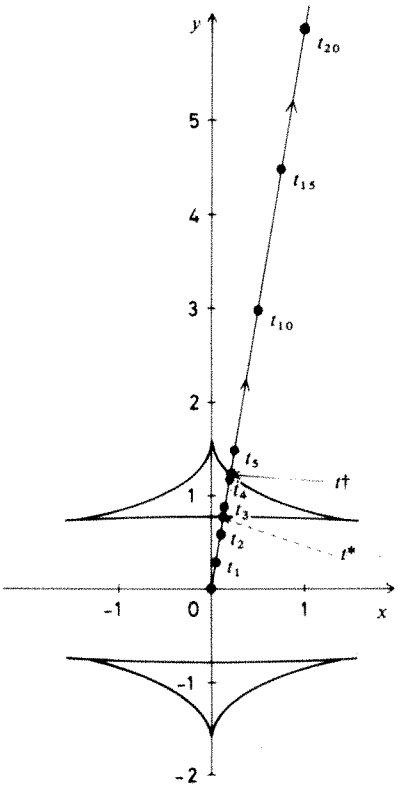


Fig. 4 Motion of the observer in the (x, y) plane ($\gamma = 1.15$) for the case discussed as an example in the present investigation. The direction of the observer's motion is indicated by an arrow.

Flux values

Since the images discussed above will have very small angular separations ($\approx 10^{-5}$ arc s) we shall mainly be interested in the total observed flux F_T from all the images

$$F_T = \sum_{i=1}^{N} F_i, \qquad N \leqq 4 \tag{10}$$

Here F_i is the observed flux from image i. In analogy with equation (4), but now using equations (1) and (2) instead of equations (1a) and (2a) one gets for a point source

$$F_i = J^{-1}(\xi_i, \eta_i) F_0 \tag{11}$$

As the light rays from B pass closer to the galaxy (that is pass through a denser star field) than those from A, the chance of getting a large disturbing effect from a star should be greater for B than for A. We therefore concentrate on B ($\gamma = 1.15$).

Figure 3 shows the main features of the function $F_T(x, y)/F_0'$. We see that a high flux region (star causes more than +100%) is found on and close to the critical curves, whereas a low flux region (star causes a reduction of 50% or more) is found in an extended region around the x-axis. The high F_T-values close to the critical curve ($J = 0$) were, of course, to be expected, since a vanishing of the jacobian for at least one of the images means an infinite flux (within the point source and geometrical optics approximation). For sources which are small but finite, one can show that the maximum value of F_T which occurs when an observer passes a critical line is proportional to $D^{-1/2}$, where D is the diameter of the source. Note that the same type of dependence was found for the spikes discussed by Benson and Cooke[13]. The large low flux region obtained in the present calculation does not occur in the case of a single point mass lens ($\gamma = 0$) discussed extensively elsewhere[4,7]. Observers within 0.75 distance units from the origin would even find that the flux is reduced by more than 90%. This is in striking contrast to the single point mass case as then a high flux region is found close to the origin. The region with 100% increase or more reaches for instance 0.55 distance units out from the origin in the (x, y) plane.

Motion in the observer's plane

Rough estimates of the transverse velocities (normal to QG) of Q, S (ref. 2) and the observer lead to an observer's velocity relative to P ($x = 0$, $y = 0$) of about 1,000 km s^{-1}. The unit of distance in the observer's plane which was chosen to be $r_0 \cdot L/(L - \lambda)$ depends on the redshifts, the Hubble parameter H_0 and slightly on the cosmological model. For galaxy redshifts

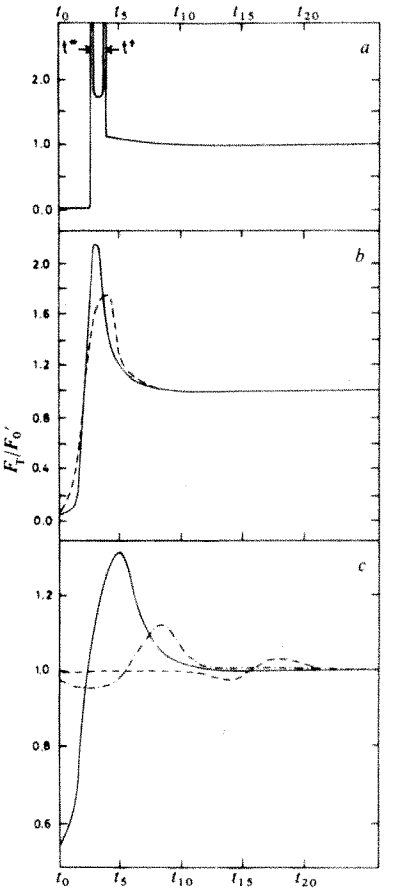


Fig. 5 Light curves with different values of D'. The real diameter of the source object Q is given by $D' r_0 L/\lambda$. *a*, Point source; *b*, $D' = 0.5$, – – – $D' = 1.0$; *c*, — $D' = 2.0$, –·– $D' = 4.0$, – – – $D' = 10.0$.

$z_G \approx 0.5$ and $H_0 = 60$ km s^{-1} Mpc^{-1} we find approximately

$$r_0 \cdot L/(L-\lambda) = 0.1 \text{ lt yr} \quad (12)$$

a value which we shall now adopt. A motion amounting to one distance unit in the observer's plane (relative motion between observer and P) will then take with velocity 1,000 km s^{-1} about

$$\Delta t = 30 \text{ yr} \quad (13)$$

An example of a motion in the observer's plane is shown in Fig. 4. Starting at time $t = t_0$ at the origin, the motion at constant velocity is followed through times $t_1 = t_0 + 0.3\,\Delta t$, $t_2 = t_0 + 0.6\,\Delta t$ till $t_{20} = t_0 + 6\,\Delta t$.

Point source

The change in the total flux F_T is demonstrated in Fig. 5*a* for the case of a point source. It is seen that $F_T(t)/F'_0$ starts out as being very small and stays <0.1 until the critical curve is reached at time $t = t^*$. Then an abrupt rise to very high values occurs (infinite by geometrical optics approximation). Very close to a critical curve (on the inside) the value of F_T/F'_0 is proportional to $d^{-1/2}$, the constant of proportionality being ~ 1 if the distance d to the critical line is expressed in normalised units. Therefore one has a minimum in F_T before the next crossing of the critical curve at $t = t\dagger$, then one again finds very high F_T values. An abrupt reduction to values $F_T/F'_0 \approx 1.2$ outside the critical curve follows. The positions of the different images in the (ξ, η) plane (on the sky) is demonstrated in Fig. 6. Starting with two images on the ξ axis one can see that a 'new' very bright image appears at $t = t^*$ and that this image immediately splits into two images. One of these images then merges with one of the original images at $t = t\dagger$ and the joint image disappears.

Extended sources

We now consider circular sources without limb darkening and with various diameters D. The source centre is in Q and the observer motion is the same as for the point mass case. The time dependence of F_T/F'_0 is shown in Fig. 5*b* and *c* for various values of D'. D' is the diameter of the source transformed into the observer's plane (just as the point source is transformed into point P) in normalised units. It is then easily seen that the diameter of the source is

$$D = D' r_0 L \lambda^{-1} \approx 0.05 D' \quad \text{lt yr} \quad (14)$$

We have used $H_0 = 60$ km s^{-1} Mpc^{-1} and $M = 1 M_\odot$. For the most reasonable cosmological models ($0 < q_0 < 1$) and redshifts z_G ($0.3 < z_G < 1$) equation (14) is accurate to ±40%.

As expected we find that the amplitudes of the flux variations get smaller and the time scales larger when D' increases. By the largest D' value shown in Fig. 5 ($D' = 10$) we still see, however, that a 5% increase in ~30 yr is possible. If we consider flux variations of <1% as insignificant we find that significant flux variations can only occur for $D' < D'_{crit} = 20$. From equation (14) we see that this corresponds to

$$D < D_{crit} = 1 \quad \text{lt yr} \quad (15)$$

Stars as deflectors can therefore only give significant flux changes for D smaller than about 1 lt yr.

As for the position and appearance of the extended images, we note that a large increase in the flux of an image is always connected with a large deformation, and that the separate images which appear of a point source often smear together for large D' values.

For small D' values the rapid flux changes when the observer crosses a critical line is of utmost interest. We have found that the maximum value of F_T/F'_0 when crossing a critical line is usually about ($D' \lesssim 0.1$)

$$F_T/F'_0 \approx 2/\sqrt{D'} \quad (16)$$

The time scale for the disappearance or appearance of the bright images is

$$\delta t = D' \Delta t \quad (17)$$

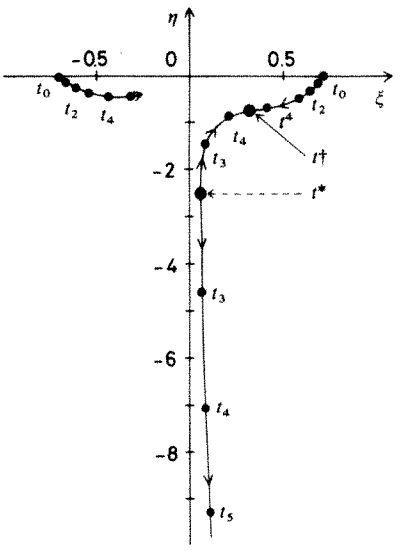


Fig. 6 Motion of the images in the deflector plane.

The very rapid X-ray fluctuations (we think intrinsic) of some quasars (time scale some hours), which have been reported from the Einstein satellite, do indicate that the D-value of the X-ray source is less than 5×10^{-4} lt yr; that is $D' < 0.01$. Large changes in F_T caused by the lens effect could then occur within some months.

Probability considerations

We have seen that significant changes in the total flux F_T can occur for sources less than about 1 lt yr, if a star happens to be close enough to the light path. The probability that such flux changes will actually occur depends on the number density of stars in the deflector plane. For simplicity we consider all stars to have one solar mass and to be lying in the deflector plane. One can then easily show that the average number density $\langle n \rangle$ of stars out to a distance GS from G is $\langle n \rangle = \pi^{-1}$ per normalised area (r_0^2). We have here made use of equation (3) for the whole galaxy, that is, with M_G instead of M_S. We can then write the number density of stars close to the light bundle as

$$n = \frac{1}{\pi} \frac{n}{\langle n \rangle} \quad (18)$$

The number density of projection points P in the observer's plane (per normalised area $r_0^2 L^2/(L-\lambda)^2$) is

$$n' = (F'_0/F_0) \cdot n = \frac{3}{\pi} \frac{n}{\langle n \rangle} \approx \frac{n}{\langle n \rangle} \quad (19)$$

as the lens effect of the galaxy reduces the projected area in the observer's plane with a factor F'_0/F_0. Noting that the low flux region in the observer's plane ($F_T < 0.5 F'_0$) has an area of 12 it is clear that the probability of large flux changes is significant even for $n/\langle n \rangle$ values as small as 0.01. We consider more likely values of $n/\langle n \rangle$ to be ~0.1. In that case the disturbance from several stars at a time would usually have to be considered.

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The heat flow field in mainland UK

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Belts of relatively high flux correspond to zones of crust enriched in radioactive heat production to ~15 km depth. The enrichments were introduced into the crust by granitic magmas.

MOST of the ~80 measurements of heat flow in the mainland UK[1-5] have been made in vertical or near vertical holes between 200 and 500 m deep from which both thermal conductivity and equilibrium thermal gradient information have been obtained. Data available up to October 1977 are summarised elsewhere[1-5], since when 26 more values have been obtained.

Although the distribution of observations is much less uniform than desirable and is determined largely by the availability of suitable boreholes drilled by other bodies, it is clear that heat flow varies in a more or less systematic manner by as much as a factor of three, between different parts of the country. Figure 1 shows the distribution of observations, and contours for 45, 60 and 75 mW m^{-2}. The contours are drawn by hand on the basis of the observations shown, without reference to any additional constraints. There are clearly different, equally possible placings for the lines shown, but from most of these emerge the broad regional patterns. A zone of relatively high heat flow extends northwestwards across central and northern England and may continue into southern Scotland (probably terminating in the Scottish Midland Valley). A second, less well defined, belt runs from Cornwall in extreme south-west England eastwards along the south coast of the country. A relatively low heat flow area occupies the western English Midlands and much of central and north Wales. The few values of north of the Scottish Midland Valley are all rather low.

Although a much better distribution of observations is required to substantiate this pattern, the observation density makes this one of the most thoroughly studied areas of its size in the world, and patterns of heat flow variation on this scale have not previously been described.

The average heat flow for the whole country is close to 60 mW m^{-2} (see Fig. 1), the lowest value recorded being 25 mW m^{-2} and the highest 130 mW m^{-2}. The precise value for the mean depends on the method used to derive it, but clearly the country is on average not notably different from other stable continental areas[6-8]. The average heat flow is, for example, close to the European average[9], 64 mW m^{-2}, and in general the values found in the UK are between the low regional flux of Scandinavia and the higher flux of central and southern Europe.

Correlation of heat flow pattern with other features

Perhaps the most striking feature of Fig. 1 is the relatively poor correlation between the heat flow pattern and other regional geophysical features. Although not presented here, maps of bouguer gravity and aeromagnetic anomalies were prepared as overlays to the heat flow map; the former shows no obvious regional correlation at all, while there is a weak correlation between the trends of magnetic anomalies and the trends of the two belts of higher heat flow values.

There is similarly little correlation with the surface geology. A weakly folded cover of Mesozoic rocks conceals the detail of the folded Palaeozoic and Precambrian basement over much of England[10-12]. In south-west England the zone of higher heat flow corresponds approximately to the trend of the Hercynian Fold Belt characterised by folded slates cut by granitic intrusives; the heat flow contours appear to close eastwards as the belt is buried by the Mesozoic cover.

The northern England zone of higher heat flow apparently cuts across the sedimentary and tectonic features of the Upper Palaeozoic basement, and the Lower Palaeozoic is very poorly known except from isolated outcrops and boreholes. The zone does, however, correspond to the subsurface trend of a hypothetical belt of folded Lower Palaeozoic rocks recently proposed by Evans[13].

The ill-defined low heat flow area contained between the two higher zones, corresponds in its eastern part to a region believed to be largely underlain by Precambrian rocks but extends westwards into north and central Wales where folded Lower Palaeozoic rocks occur at the surface.

Compilations of continental heat flow show that there is a broad dependence of values on the age of the last thermal event to have affected the crust[7,14-17]. The majority of the values shown in Fig. 1 come from crust which is in this sense 400–250 Myr in age and exhibit no correlation with the age. The variation of heat flow reported here must thus represent the considerable scatter found in the broad-scale compilations.

Origin of the variation in surface heat flow

The fact that the mean heat flow for the country as a whole is not exceptional, together with the scale width of the variation in surface heat flow, suggest that the origin of the pattern is not to be found in some unusual condition in the mantle but rather reflects some crustal condition or process[18-20].

Even very slow and low-volume flows of water in the crust may transfer amounts of heat by convection which are of the same order as the vertical conductive flux[2,21]. Such flows must, however, be driven either by a topographic head, or by initial horizontal temperature differences. Viewing the regional heat flow pattern as a whole there is no obvious correlation with topography or with the known pattern of near surface groundwater movements[22]. On the other hand, there are almost certainly areas where water movements do control the heat flow on a local scale (for example, the limestone area of south Derbyshire[1]).

The thermal conductivity of rocks commonly varies by a factor of two between different lithologies[2,3]. A crust characterised by adjacent belts of high conductivity and low conductivity rocks would be expected to refract any uniform heat flux supplied to it from below, so that the higher conductivity regions carried a higher heat flow, at any rate near their margins[24,25]. We have considered the possibility that the high heat flow belts of Fig. 1 are formed in this way and simple two-dimensional analytical and numerical modelling has been carried out. We find that steep sided zones with twice the conductivity of their surroundings, and extending to a depth of half, or even the full thickness of the crust, show strong refractive edge effects which perturb the surface heat flow by $\sim \pm 20\%$. This would be just sufficient to explain the amplitude of the observed variation but the sharp depressions and elevations of heat flow at the junction of the conductivity domains do not match the observed broad areas of low flux and intervening belts with distinctly higher flux throughout. Despite these general considerations there is little doubt that on a local scale refraction of heat flow is important[26].

In regions which have not experienced recent igneous or metamorphic activity a significant fraction of the surface heat

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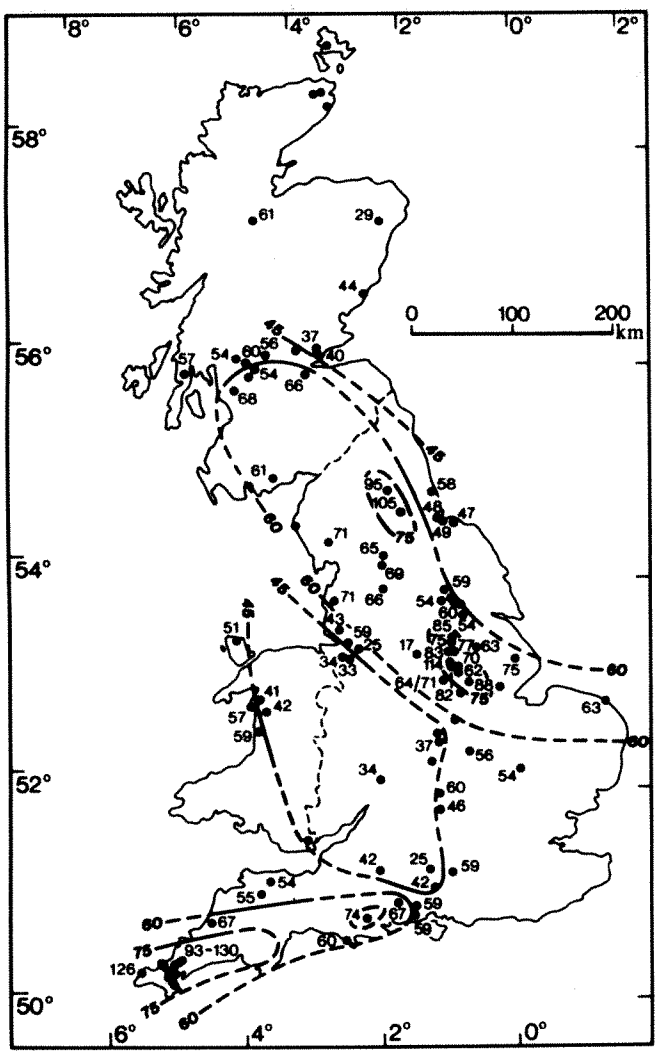


Fig. 1 Heat flow sites with the values contoured at 45, 60 and 75 mW m^{-2}. Although uncertainty attends the placing of the contours, particularly across the Scottish Southern Uplands and in Wales, the two belts of higher values stand distinctly above background.

flow of continents is derived from heat producing isotopes which are strongly concentrated in the continental crust with respect to mantle abundances. At equilibrium, the surface heat flow may be expressed as:

$$q_0 = q_m + \int_0^m A\,dz \qquad (1)$$

where m refers to the depth (z) above which enrichment is both significant and variable; q and A refer to heat flow and heat productivity respectively. Studies of several continental regions[27–29] suggest that the integral in equation (1) can be solved simply as A_0D on the empirical grounds that q_0 and the surface heat production are linearly correlated in regions where high grade gneissic, or granitic intrusive rocks outcrop. The parameter D in these terrains has a characteristic value of 8.0 ± 1.5 km and q_m accounts for about half the average heat flow observed[29]. We have previously reported heat flow and productivity measurements for southern Britain[1] which showed good correlation where either granitic or low grade cleaved metasediment or metavolcanic rock outcrop, or are encountered as basement in boreholes. These and further data for Britain, including southern Scotland, are plotted in Fig. 2 which support the correlation ($R = 0.96$); D has a value of 16.6 km. This relationship implies that the granitic and weakly metamorphic basement of Britain, whether outcropping at the surface or buried beneath a platform of younger sediment, is enriched in heat production to depths of ~15 km and that the degree of enrichment is geographically variable and causes the heat flow variation.

Although, in principle, such an explanation could account for the large scale features of Fig. 1, it does not explain how such variation in heat production could have come about. We have argued elsewhere[1] that much of the basement to these depths comprises slates and other low grade metamorphic rocks of sedimentary origin. Thus perhaps the simplest explanation of the heat flow pattern is that it maps out areas beneath which sediments of differing bulk heat production are preserved. Such differences could reflect either the provenance or the depositional environment of the sediments. However, the contours of heat flow, in northern Britain (Fig. 1) at least, cut across apparently discrete depositional zones such as the Midland Valley and Southern Uplands of Scotland[30].

All the highest values of heat flow in Britain have been measured in holes penetrating granites with high heat production and several bodies of this kind occur at the surface in the southern high heat flow belt, and shallowly in the subsurface of the northern belt. The deep structure and possible connections between these bodies has been the subject of research for some time[31–33] and a possible explanation for the high heat flow zones is that they correspond to zones of high heat-production intrusions presumably associated with old orogenic belts. Note that all the granites are too old for there to be detectable effects associated with their original cooling after employment.

The systematic variations of chemical composition of acidic and intermediate igneous suites normal to the trend of such belts have been extensively studied[34] and have generally been related to special conditions associated with partial fusion of a descending lithospheric slab[34–36]. Thus the chemical anomalies implied by the high heat flow zones could be regarded as features introduced into the crust at the time of granitic magmatism. In the present cases the southern high heat flow belt would be a Hercynian feature and the northern zone a Caledonian feature following the trend suggested by Evans[11]. If it could be demonstrated that the northern belt was indeed continuous from the Lake District across the Southern Uplands to the Midland Valley of Scotland, its formation would clearly postdate the closing of the Southern Uplands crustal suture[30].

The correlation in Fig. 2 implies some degree of communication between heat production in granitic rocks and in slates, because the same scale length for heat production distribution applies to either rock type. It is envisaged that such communication could be either physicochemical, or purely physical, or most likely some combination of the two.

A chemical communication could be set up by the interchange of fluid between granites and their country rocks. The cooling of granitic bodies may induce groundwater circulations[37,38] which are able to scavenge incompatible elements, such as those responsible for heat production, from large volumes of country rock and reprecipitate them within the granites and their immediate surroundings. Figure 2 would imply that the scale length of the convective process, and hence the fluid interchange, was ~15 km. It is, however, unlikely that redistribution of heat production could, by itself, give rise to the observed heat flow belts. The pattern expected from such a process alone would be one in which zones of elevated flux were flanked by exactly compensating zones of depressed flux resulting from hydrothermal scavenging. In view of the observed generally low heat flow outside the belts the main effect of the granitic rocks is probably to provide initial enrichment to the belts.

A purely physical relationship between heat production distribution within granites and slates can be envisaged in a two-layer crust; the lower layer would consist of relatively dense rocks of uniform heat production within which granites are too buoyant to reside for the period of their crystallisation. The upper layer would comprise the slates themselves and their intrusive granitic stocks. On this interpretation (a similar one to that proposed by Smithson and Decker[38]) heat production in each rock type would be uniform at all depths within the upper

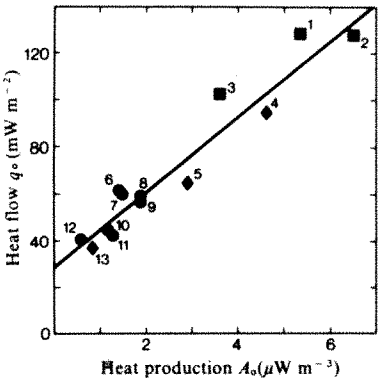


Fig. 2 Surface heat flow plotted against radioactive heat production in local granitic or low-grade metasedimentary and metavolcanic basement. Regression line: $q = 16.6A + 27$. Correlation coefficient $R = 0.96$. ■, Hercynian granites of south-west England. ◆, Lower Palaeozoic granites. ●, Cleaved phyllitic basement. Data for points, 1, 2, 4, 5, 7, 8, 9, 11, 12 and 13 are summarised in ref. 1. Point 3 is Longdowns[3]. Points 6 (Langstrothdale) and 10 (Montrose) (unpublished data). The slope and intercept of the correlation would be substantially unchanged were granitic rocks alone to be considered.

layer; the slope of the correlation in Fig. 2 is thus directly interpreted as the mean thickness of the upper layer.

Conclusions

We conclude that the main features of the heat flow field in mainland UK reflect lateral variations in the concentration of heat producing elements within the upper half of the crust. These in turn arise from the distribution of high heat-production granitic bodies which introduced heat production anomalies into an upper crust which was initially predominantly slaty in character. Although now cool the granites may, at the time of their intrusion, have initiated large scale convective circulations of water within the crust, which redistributed trace elements in sedimentary and igneous rocks alike. Enrichments in heat production might be expected to sustain some low-volume circulation over long periods of time. On a local scale the heat flow field is modified both by present day water flows and by heat flow refractions.

The relatively long scale length of the heat production distribution implies that near-surface temperature gradients in, for example, the Cornish granites should extend virtually unmodified to depths of several kilometres. Note that the same processes which redistribute heat-producing elements might also be expected to concentrate other elements in ore deposits. There is a weak correlation between the high heat flow areas and exposed zones of epigenetic mineralisation. This correlation may extend into areas where older rocks are hidden beneath a cover of Mesozoic and younger sediments.

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Organisation and sequence studies of the 17-piece chicken conalbumin gene

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The conalbumin gene has been cloned and shown to consist of at least 17 exons approximately 60–200 base pairs long. The DNA sequence upstream from the region coding for the 5′ end of the mRNA shows similarities with sequences present in homologous positions in other genes. High and low frequency repetitive sequences are found both upstream from the conalbumin gene and within one intron.

CONALBUMIN (or ovotransferrin) is one of the major egg white proteins synthesised by the tubular gland cells of the magnum portion of hen oviduct. Its expression is controlled at the transcriptional level by oestrogens and progesterone, as are the other egg white proteins (see ref. 1 and refs therein). Nevertheless there are some differences in the patterns of expression of these genes after hormone administration. For instance, oestradiol administration results in a very rapid increase of conalbumin mRNA (con-mRNA), whereas the appearance of ovalbumin mRNA is somewhat delayed (see ref. 2 and refs therein). Comparison of the DNA sequences flanking these genes might

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therefore provide a means of testing the idea that steroid hormones act (at least in part) through interactions of steroid–receptor complexes with the genome (see refs 3 and 4 and refs therein). In addition, the study of the conalbumin gene and its flanking sequences provides an interesting insight to the problem of tissue-specific expression of a unique gene[1].

Oviduct conalbumin and liver transferrin are the same protein, differing only in the nature of their carbohydrate moiety[5,6]. However, the modulation of the expression of the conalbumin-transferrin gene is very different in the two tissues. The expression of the oviduct gene is much more sensitive to oestradiol administration than is that in the liver, despite the presence of oestradiol receptors in chicken liver[7]. In addition, iron levels appear to be involved in regulating the expression of the gene in the liver, but not in the oviduct (G. S. McKnight, D. Lee and R. Palmiter, personal communication).

In previous studies we have constructed a recombinant plasmid containing a doubled-stranded cDNA (dscDNA) copy of con-mRNA (pBR322-con1, (ref. 8)) and have used it to investigate the structure of the conalbumin gene[1]. Restriction enzyme mapping of the genomic DNA revealed that the conalbumin gene is split and is contained in three *Eco*RI fragments, 5′-*Eco* 'b'-*Eco* 'c'-*Eco* 'a'-3′, about 4.0, 2.5 and 11.0 kilobase pairs long, respectively (see Fig. 1*A*(*b*); ref. 1) The *Eco*RI fragments 'b' and 'c' and about 1,500 base pairs at the 5′ extremity of fragment 'a' were cloned in the λ phage recombinant λC4-con1 and shown to contain the sequences coding for the first 940 nucleotides of the 2,400 nucleotide-long con-mRNA[1].

We now report the molecular cloning of the conalbumin *Eco*RI fragment 'a', which allows us to describe the organisation of the complete conalbumin gene. The DNA base sequence within and upstream from the region coding for the 5′ end of con-mRNA has also been obtained and the existence of repeated sequences within the conalbumin gene and upstream from its 5′ end is reported.

Cloning of conalbumin *Eco*RI fragment 'a'

Since previous attempts to isolate the complete *Eco*RI fragment 'a' from libraries representing the whole chicken genomic DNA had been unsuccessful[1], we decided to start from a DNA fraction enriched in this fragment. A recombinant, λWconA1, hybridising to a probe specific for fragment 'a', was isolated as outlined in the legend to Fig. 2. Digestion of this cloned DNA with *Eco*RI revealed the presence of a DNA fragment of a similar size to that of the genomic conalbumin *Eco*RI fragment 'a' (Fig. 2, lanes 1 and 2). Comparison (Fig. 2) of the restriction enzyme patterns obtained from the cloned and the partially purified cellular fragment 'a' digested with *Xba*I (lanes 3 and 4), *Bam*HI (lanes 5 and 6), *Bgl*I (lanes 8 and 9) and *Bgl*II (lanes 10 and 11), confirmed that the cloned fragment is indeed the conalbumin *Eco*RI fragment 'a'.

Combined with unpublished and previously reported mapping[1], the present restriction enzyme digestions allow the construction of the conalbumin gene restriction enzyme map shown in Fig. 1*A*(*b*). In addition, we have used (results not shown) the con-dscDNA probes, *Eco*A and *Eco*B (Fig. 1*B*(*a*)), *Eco-Bgl*I, *Eco-Bgl*II and *Eco-Bam*1 to localise, on the genomic DNA map, the *Eco*RI, *Bgl*I, *Bgl*II and *Bam*HI sites of con-dscDNA (Fig. 1*A*(*a*), (*b*)). The *Bam*2 site of the dscDNA (Fig. 1*A*(*a*)) was not found in the genomic clone. This discrepancy is accounted for by the existence of allelic variants for this *Bam*HI site (our unpublished results and D. Lee, G. S. McKnight and

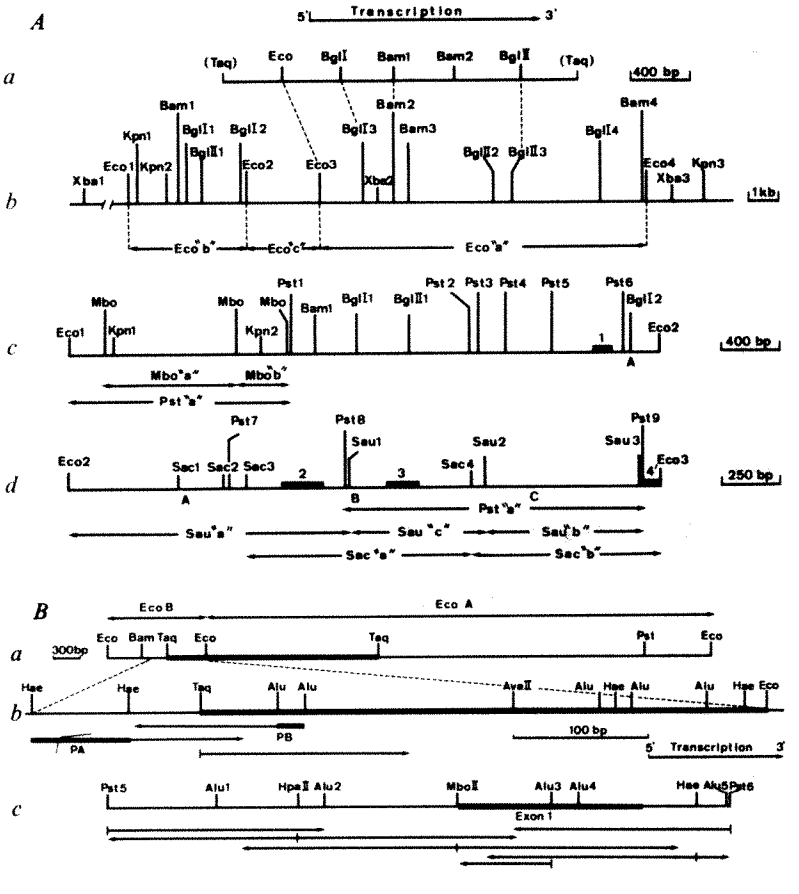


Fig. 1 *A*, Organisation of the sequences coding for conalbumin mRNA in chicken erthrocyte DNA and in genomic DNA clones. *a*, Partial restriction endonuclease map of the dscDNA copy of con-mRNA and scale in base pairs (bp) (taken from ref. 8). *b*, Restriction endonuclease map of the erythrocyte DNA region containing the conalbumin gene and scale in kilobase pairs (kb). This map extends from that previously published[1] to include the *Bam*HI, *Bgl*I and *Bgl*II sites. These sites were mapped (our unpublished results) using the previously cloned *Eco*RI fragment 'b' (ref. 1) and the presently cloned *Eco*RI fragment 'a' (see text and Fig. 2). The sequences coding for con-mRNA are contained in *Eco*RI fragments 'a', 'b' and 'c'[1]. The dotted lines between *a* and *b* indicate the correspondence between the restriction enzyme sites found in the dscDNA and the genomic clones (see text). *c*, Restriction endonuclease map of the cloned *Eco*RI fragment 'b' and scale in bp. For each restriction enzyme all of the existing sites are shown except for *Mbo*II where only the relevant sites are shown (see text and Fig. 6). The number 1 corresponds to exon *1* and A represents the 5′ moiety of intron *A*[1], *d*, Restriction endonuclease map of the cloned *Eco*RI fragment 'c' and scale in bp. A, B, C, 2, 3, and 4′ are the 3′ moiety of intron *A*, intron *B*, intron *C*, exon *2*, exon *3* and the 5′ moiety of exon *4*, respectively[1]. ***B*, Strategy for DNA sequencing at the 5′ end of conalbumin double-stranded cDNA and around exon *1* in the cloned genomic DNA.** *a*, Partial restriction enzyme map of pBR322-con1[8] linearised with *Eco*RI and scale (in bp). The heavy line corresponds to the inserted con-dscDNA. The two *Taq*I sites were generated during the insertion of the con-dscDNA in the *Sal*I site of pBR322[8]. *Eco*A and *Eco*B correspond to the fragments which are generated by *Eco*RI digestion of pBR322-con1[1,8]. *b*, DNA sequencing at the 5′ end to con-dscDNA and scale (in bp). The pBR322-con1 fragment *Eco*B (see *a*) was mapped by digestion with various restriction enzymes either singly or in combination and the restriction enzyme map of a selected region is represented. Due to the small size of the fragments resulting from *Alu*I digestions, it was necessary to label them at their 5′ end with [γ-^{32}P]-labelled ATP and T4 polynucleotide kinase in order to reveal them by autoradiography. Primers *A* and *B* (*PA* and *PB*) were prepared by acrylamide gel electrophoresis of *Alu*I or *Hae*III digests of purified fragment *Eco*B[1]. They were used to sequence around the *Taq* site by using the chain termination method of Sanger *et al.*[10] and the extent of the sequenced regions are shown by the arrows. The third arrow indicates the extent of the region sequenced from the *Taq* site using the method of Maxam and Gilbert[11] (*Taq-Eco* fragment end-labelled with [γ-^{32}P]-labelled ATP and cut with *Ava*II). *c*, DNA sequencing within and around exon *1* of the genomic DNA. The *Pst*5-*Pst*6 fragment of *Eco*RI fragment 'b' (1*Ac*) was subcloned in pBR322, isolated on sucrose gradient after *Pst*I digestion and mapped with various restriction enzymes as outlined under *b*. DNA sequencing was carried out using the Maxam and Gilbert method[11]. The arrows indicate the direction and the extent of the sequence determinations from the 5′ end-labelled restriction enzyme sites. When a given region was not sequenced on the two strands, it was sequenced at least twice on the same strand to ensure the accuracy of the sequence determination. The abbreviations used for restriction enzymes are for *Eco*RI, *Bam*HI, *Taq*I, *Pst*I, *Hae*III and *Alu*I. Sequencing gels and additional details about the strategy are available upon request. Scale as in Fig. 1*B*(*b*).

R. Palmiter, personal communication) similar to those which were previously described for the ovalbumin gene[9,12-15].

13 introns are present in the conalbumin *Eco*RI fragment 'a'

Seven conalbumin exons (*1–7*) separated by six introns (*A–F*) have previously been localised in *Eco*RI fragments 'b' and 'c' and in the previously cloned 1,500 base pair long 5′ segment of fragment 'a' (see ref. 1, and Figs 1*A*(*b*) and 4*f* and *g*). The cloning of fragment 'a' allows one to determine whether the ~2,000 nucleotides of con-mRNA which are not encoded in *Eco*RI fragments 'b' and 'c' are completely encoded in fragment 'a'.

Electron microscopy of hybrid molecules formed between con-mRNA and the cloned genomic *Eco*RI fragment 'a' reveals that con-mRNA hybridises to *Eco*RI fragment 'a' over 2,048 ± 184 nucleotides in 14 exonic RNA–DNA hybrid regions (*4″–17*), separated by 13 intronic single-stranded DNA loops (*D–P*) (Figs 3*A* and 4*b*). As expected from our previous results[1] the length measurements show that the first 1,500 base pairs at the 5′ extremity of fragment 'a' contain the previously identified introns *D*, *E* and *F*, the 3′ moiety of exon *4* and exons *5* and *6*. These observations definitely prove that the conalbumin *Eco*RI fragments 'c' and 'a' are contiguous. The organisation and the length of the various exonic and intronic sequences present in *Eco*RI fragment 'a' are given in Fig. 4*b*, *g*.

There is excellent agreement between the restriction enzyme map and the electron microscopic map. From the comparison of these two maps (Fig. 4*a*, *b*) one can predict that the restriction sites *Bam*2, *Bam*3 and *Bgl*II2 should be located in exon *9*, intron *I* and intron *N*, respectively. These predictions were verified by hybridising con-mRNA with specific restriction enzyme segments (Fig. 3). For instance, hybridisation with the *Eco*3-*Bam*2 and the *Bam*3-*Bam*4 fragments (Fig. 4*a*) allows one to localise *Bam*2 and *Bam*3 sites in exon *9* and in intron *I*, respectively (Figs 3*B* and 4*c*, *d*). Hybridisation of con-mRNA with *Eco*3-*Bgl*II2 fragment (Fig. 4*a*) indicates that site *Bgl*II2 is located in intron *N* (Figs 3*C*, 4*e*). Also, the *Bgl*I3 and *Bgl*II3 exonic sites (see above and Fig. 1*A*(*a*), (*b*)) appear to be located in exons *7* and *16*, respectively (Fig. 4*a*, *b*).

The conalbumin gene is in 17 pieces

From our present and previous results[1], it appears that the conalbumin gene consists of at least 17 exons separated by 16 introns (we cannot exclude the existence of additional introns too small to be detected by electron microscopy). This striking structure is visualised in Fig. 3*D* in the form of a hybrid molecule between con-mRNA and the linked cloned conalbumin *Eco*RI fragments 'b', 'c' and 'a'. Seventeen exonic RNA–DNA hybrid regions (*1–17*) separated by 16 intronic, single-stranded DNA loops are visible (Figs 3*D*, 4*f*, *g*). The length of the entire DNA–RNA hybrid region is 2,508 ± 164 base pairs, in very good agreement with our previous estimate of con-mRNA length of about 2,400 nucleotides[8]. Since there is no clearly visible tail at the 3′ end of the hybrid region (see also Fig. 3*A*, *B*) despite the existence of a poly(A) sequence attached at the 3′ end of con-mRNA, it is very likely that exon *17* contains the DNA sequence coding for the 3′ end of con-mRNA. Exon *1* contains the DNA sequence coding for the 5′ end of the mRNA (see below). It appears therefore that con-mRNA is entirely encoded in the 10.3 kb genomic region which is between the 5′ end of exon *1* and the 3′ end of exon *17*. This conclusion is supported by the observation (M. LeMeur and A. Krust, personal communication) that the longest con-mRNA precursor molecule which which can be found in oviduct RNA is 9,900–10,100 nucleotides long.

The 5′ end of conalbumin mRNA is encoded in exon *1*

Since the sequence of con-mRNA was totally unknown and we previously showed that the con-dscDNA plasmid pBR322-con1

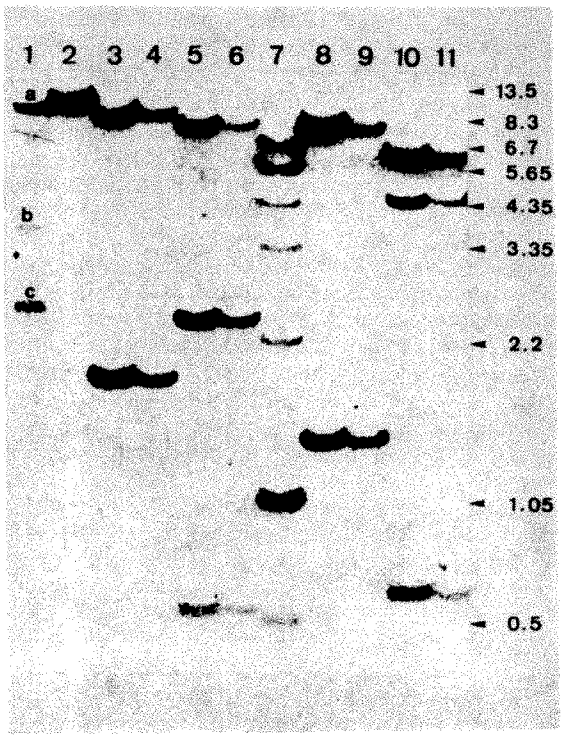
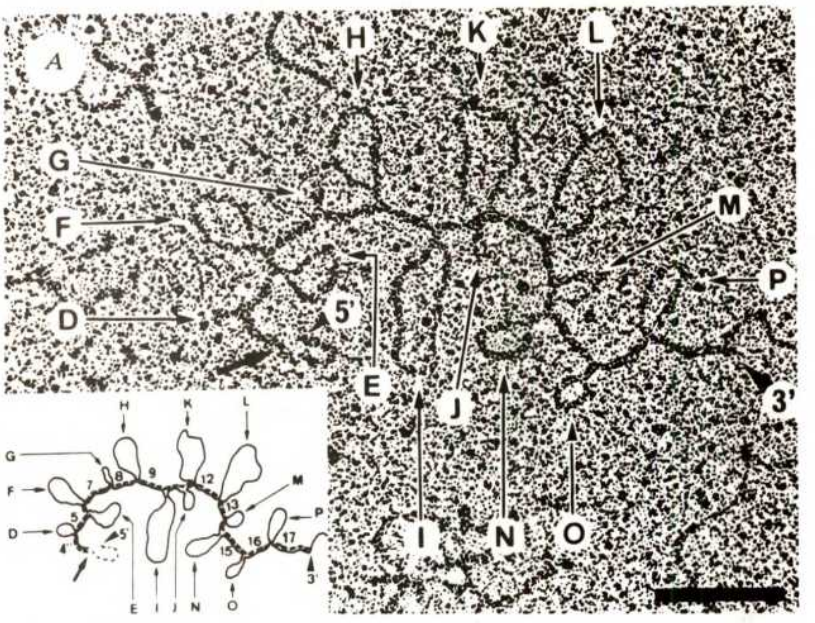
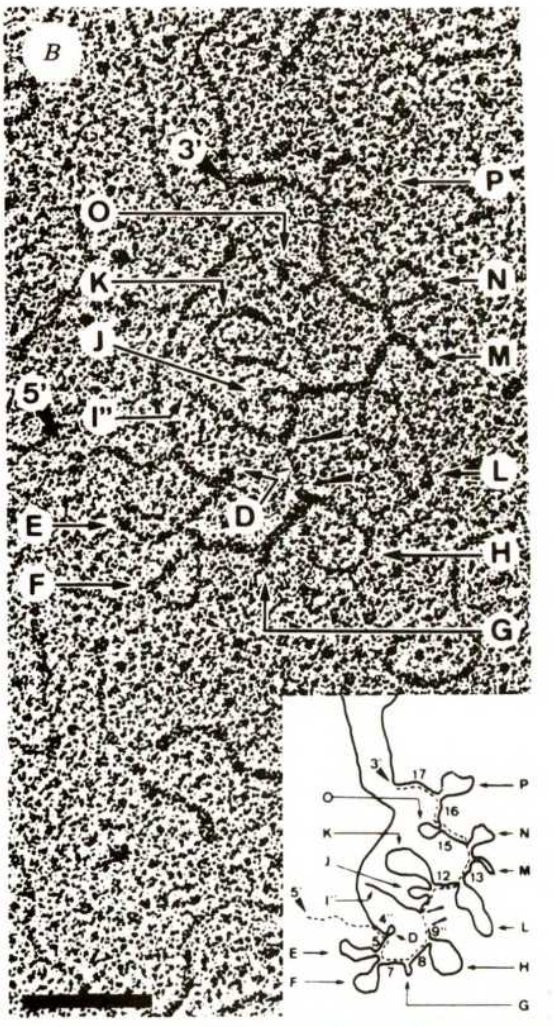


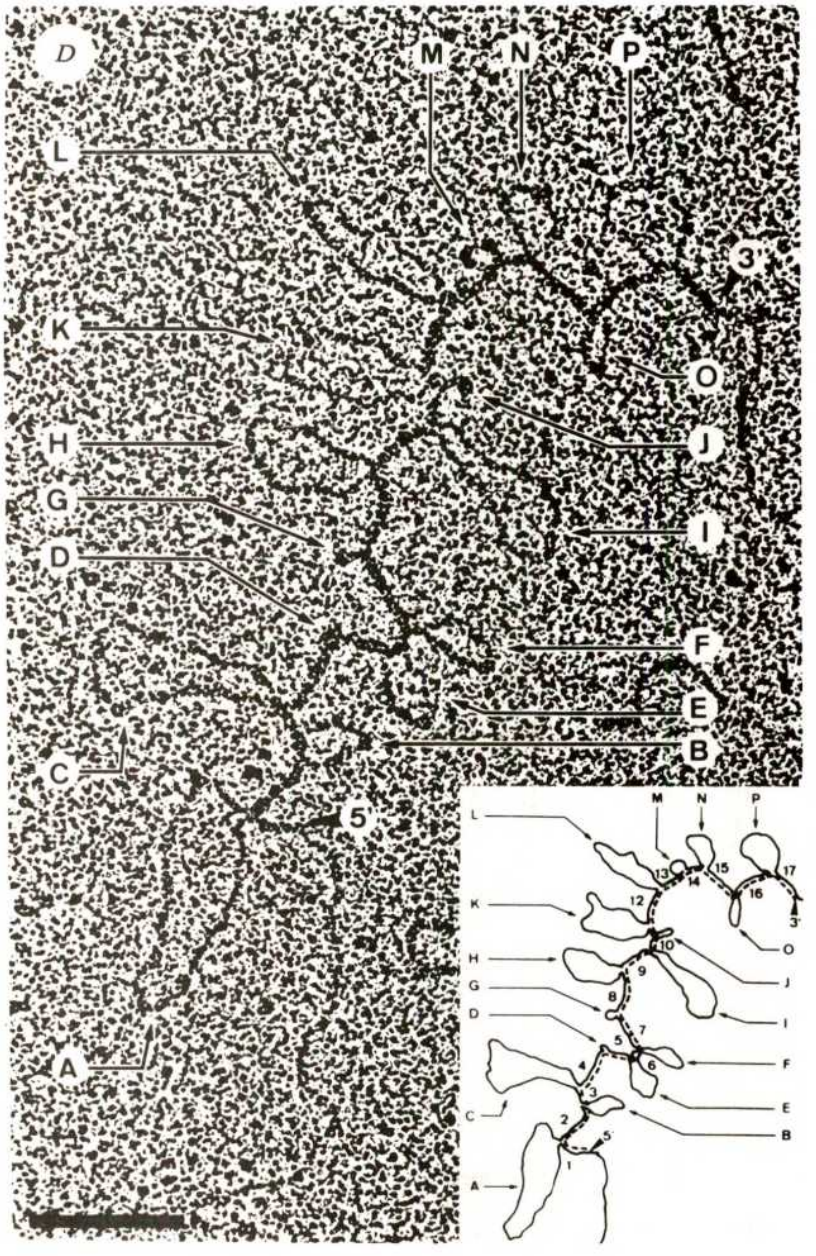
Fig. 2 Comparison of restriction enzyme fragments obtained from the cloned and partially purified cellular *Eco*RI fragments 'a'. The cloned *Eco*RI fragment 'a' was obtained as follows: 0.25 μg of an *Eco*RI digest of erythrocyte DNA partially purified by two cycles of RPC-5 chromatography[9] and sized by sucrose gradient centrifugation, was ligated with 0.6 μg of λgtWES.λC right and left arms and subsequently packaged and cloned as previously described by Garapin *et al.*[16] using the conalbumin dscDNA probe specific for the sequences coded for by *Eco*RI fragment 'a' (*Hha* B probe[1]). Out of 10,000 recombinants which were screened, one (λWconAI) hybridised to the probe and was amplified. About 2 μg of RPC-5 partially purified erythrocyte DNA (see above) (lanes 3, 5, 8, 10) and 0.3 ng of an *Eco*RI digest of λWconA1 DNA (lanes 4, 6, 9, 11) were digested with *Xba*I (lanes 3 and 4), *Bam*HI (lanes 5, 6), *Bgl*I (lanes 8 and 9) and *Bgl*II (lanes 10 and 11), electrophoresed on a 1% agarose slab gel, transferred onto nitrocellulose filters, hybridised with ^{32}P-labelled con-dscDNA probe pBR322-con1[8] and revealed by autoradiography as previously described [1,17]. Lanes 1 and 2 contained an *Eco*RI digest of total erythrocyte DNA (15 μg) and λWconA1 DNA (0.3 ng), respectively. ('a', 'b' and 'c' indicate the position of the three genomic *Eco*RI fragments.) Lane 7: hybridising size markers derived from pBR322-con1 plasmid. The size of the hybridising fragments (in kilobases) are: Lane 1: (a) 11, (b) 4, (c) 2.5; lane 2: 11; lanes 3 and 4: 9.1 and 1.9; lanes 5 and 6: 8, 2.45 and 0.55; lane 7: 6.7, 5.65, 4.35, 3.35, 2.2, 1.05 and 0.5; lanes 8 and 9: 7.9 and 1.45; lanes 10 and 11: 5.8, 4.6 and 0.6.

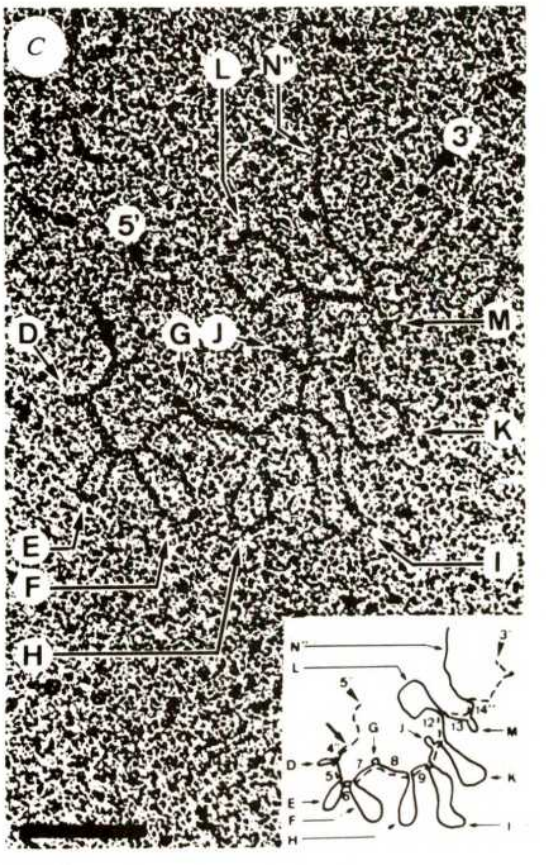
[Fig. 1*B*(*a*)] should contain most of the sequences corresponding to the 5′ end of the mRNA[8], we first sequenced the 5′ end of con-dscDNA. The sequence shown in Fig. 5*A* was obtained using the Maxam and Gilbert[11] and Sanger *et al.*[10] methods as outlined in the legend to Fig. 1*B*(*b*). The first possible AUG initiation codon is found 57 base pairs downstream from the 5′ end of the dscDNA. This AUG codon is the initiation codon for conalbumin, since it is followed (with one exception, see below) by the sequence coding for the 19 amino acids present at the N-terminal of pre-conalbumin (protein leader sequence, or signal peptide[6]) and the first amino acid (alanine)[6,18] of mature conalbumin. There is one difference at position −2 of the leader sequence of amino acids between our version derived from DNA sequencing and that of Thibodeau *et al.*[6] obtained by amino acid sequencing. This discrepancy does not seem to be related to DNA or protein sequencing inaccuracy (R. Palmiter, personal communication) and may reflect the existence of two allelic variants of the conalbumin gene similar to those mentioned above for *Bam*HI site.

Since it was very likely that the DNA sequence corresponding to the 5′ end of the dscDNA would be found in the genomic exon *1*, we sequenced the *Pst*5-*Pst*6 fragment of *Eco* 'b' which


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5'
I''
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C
5'
A


C
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N''
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contains exon *1* [Fig. 1*A*(*c*) and *B*(*c*)]. The sequence is presented in Fig. 5*A*. As expected, we found a region where the genomic and the dscDNA sequences were identical. This region, which contains the *Alu*3 and 4 sites, codes for the first 14 amino acids of the protein leader sequence. The limit between exon *1* and intron *A* is given by comparing the cellular and the dscDNA sequence (dashed line). The exonic–intronic junction sequence resembles the model sequence[19] which was derived by comparing several exon–5′-intron junction sequences and, once again, the dinucleotide GT is present at the 5′ end of an intron, in agreement with the 'GT-AG rule'[19].

Although the above results confirmed that exon *1* contains sequences coding for the 5′ region of con-mRNA, sequencing the 5′ end of the mRNA was necessary to determine the location of the 5′ extremity for exon *1* and whether it contains the sequence coding for the 5′ end of the mRNA. The sequence of the 5′ end of con-mRNA was obtained by using the genomic *Alu*3-*Alu*4 fragment [see Fig. 1*B*(*c*)] as a primer and the modified chain termination method of Sanger *et al.*[10,20] in order to sequence reverse transcripts of mRNA (see legend to Fig. 5*B*). With the exception of the first nucleotide, the sequence of the first 60 nucleotides of the mRNA was obtained and is presented in Fig. 5*A*. A sequencing gel corresponding to the first 36 nucleotides is shown in Fig. 5*B*. The first nucleotide of the mRNA cannot be determined with this method and the band which is present in all four lanes (lanes 1–4 of Fig. 5*B*) above the first U corresponds presumably to the first nucleotide of the mRNA as indicated by the position of an [*Alu*3-*Alu*4]-primed reverse transcript of the mRNA synthesised in the absence of dideoxynucleoside triphosphates (Fig. 5*B*, lane 5). The first nucleotide of the mRNA should therefore be an A by comparison with the cellular DNA sequences shown in Fig. 5*A*. The nature of the first nucleotide of the mRNA was confirmed as follows: an *Alu*2-*Alu*3 fragment end-labelled with [γ-^{32}P]-labelled ATP and polynucleotide kinase, was hybridised to con-mRNA under conditions which favour DNA–RNA hybrid formation[21], digested with S_1 nuclease, and electrophoresed on an acrylamide gel under denaturating conditions in parallel with the chemically cleaved products for DNA sequencing according to Maxam and Gilbert[11] of the same region. The migration of the major S_1-protected fragment corresponds to the T, which in the coding strand of the genomic sequence, is complementary to the A numbered 1 in the non-coding strand (Fig. 5*A*) (R. Hen, unpublished result). The first nucleotide of con-mRNA is therefore probably an A. However, direct RNA sequencing will be required to definitely establish the 5′ end sequence of con-mRNA. From the present study we conclude that exon *1* contains the sequence coding for the 5′ end of con-mRNA and that there is a 76 nucleotide-long untranslated region at the 5′ end of con-mRNA. Therefore con-mRNA, in contrast to ovalbumin and some viral mRNAs (see refs 19 and 24 and refs therein), but like other eukaryotic mRNAs, do not have an untranslated leader sequence encoded in a separate exon at its 5′ end.

Presence of repetitive sequences within and around the conalbumin gene

None of the conalbumin mRNA-coding sequences is repeated in the chicken genome[1]. Studies on the ovalbumin[9,16] and globin[22] genes have shown that the intronic sequences of these genes are also unique. In contrast, when a restriction enzyme digest of total chicken DNA is blotted onto a nitrocellulose filter and hybridised with either conalbumin *Eco*RI fragment 'b' or 'c' labelled by nick-translation, a very fast and complete darkening of the autoradiographic film is observed (not shown). This indicates the presence of highly repetitive sequences in both the *Eco*RI fragments.

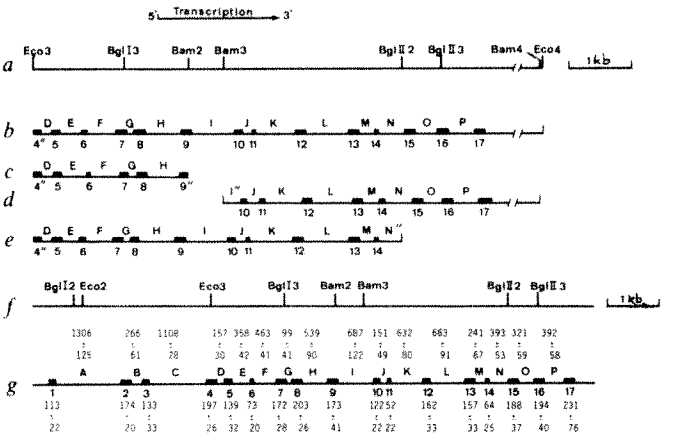


Fig. 4 Schematic representation of the hybrid molecules between con-mRNA and cloned genomic DNA fragments as measured by electron microscopy. *a*, Restriction enzyme map of conalbumin *Eco*RI fragment 'a' (see Fig. 1*A*(*b*)) and scale in kb for *a*–*e*. *b*, Localisation of exonic and intronic sequences contained in cloned *Eco*RI fragment 'a' (see Fig. 3*a*). Heavy lines (*4″* and *5–17*) refer to DNA–RNA hybrid (3′ moiety of exon *4* and exons *5–17*, respectively) regions. *D–P* correspond to single-stranded intronic loops. The length of the exonic sequence *4″* is 119 ± 29 base pairs and those of exon *5–17* are given under *g* together with the lengths of introns *D–P*. *c*, *d*, Localisation of exonic and intronic sequences within the *Eco*3-*Bam*2 and *Bam*3-*Bam*4 fragments (see Fig. 3*b*). Notation as under *b*, except for *9″* (the 5′ moiety of exon *9*) and *I″* (the 3′ moiety of intron *I*). The lengths of *4″*, *9″* and *I″* are 140 ± 22, 87 ± 44 and 296 ± 60 base pairs, respectively. The lengths of the other exons and introns are as shown in *g* within the experimental error. *e*, Localisation of exonic and intronic sequences within the *Eco*3-*Bgl*II2 fragment (see Fig. 3*c*). Notation as under *b* except for *N″* (the 5′ moiety of intron *N*). The lengths of *4″* and *N″* (in base pairs) are 113 ± 30 and 358 ± 94, respectively. The lengths of other exons and introns are as under *g* within experimental error. *f*, Restriction enzyme map of the conalbumin gene region (see Fig. 1*A*(*b*) and scale in kb for *f* and *g*. *g*, Localisation of the exons and introns in the conalbumin gene as established from the present data and those previously reported for the cloned conalbumin *Eco*RI fragments 'b' and 'c' and the 5′ moiety of *Eco*RI fragment 'a'[1]. Letters *A–P* and numbers 1–17 (heavy lines) correspond to the conalbumin gene introns and exons, respectively. The length of the exons *5–17* and of introns *D–P* are taken from the measurements made on 25 hybrid molecules similar to that shown on Fig. 3*a*, whereas the length of exons *1–4* and introns *A–C* are taken from Perrin *et al.*[1].

Fig. 3 Electron micrographs of hybrids between conalbumin mRNA and cloned genomic DNAs. The hybrid molecules were prepared as described previously[1]. In the line drawings, the dashed line represents the RNA and the solid line the DNA. Capital letters refer to intronic sequences, and numbers to the exonic RNA–DNA hybrid sequences (for the sake of clarity not all of the exons are numbered in the line drawings). The 5′ and 3′ ends of the con-mRNA molecules are indicated with arrows. ***a*, Hybrids between con-mRNA and heat-denatured cloned *Eco*RI fragment 'a' purified from λWconA1 DNA.** The RNA–DNA hybrids (2,048 ± 184 base pairs) are interrupted by 13 single-stranded DNA loops (*D–P*) (see line drawings and Fig. 4*b*). The length of the 3′ moiety of exon *4* (*4″*) and those of exons *5–17* together with the length of introns *D–P* are given in Fig. 4*b*, *g* and their legends. The unnumbered arrow points to the 5′ end of the RNA–DNA hybrid region which corresponds to site *Eco*3 (see Fig. 4*a*, *b*). ***b*, Hybrids between con-mRNA and heat-denatured *Eco*3-*Bam*2 and *Bam*3-*Bam*4 fragments of the cloned *Eco*RI fragment 'a'.** The *Eco*3-*Bam*2 fragment (Fig. 4*a*) was subcloned in pBR322 and the recombinant plasmid was linearised with *Bam*HI before heat-denaturation. The *Bam*3-*Bam*4 fragment (Fig. 4*a*) was subcloned in pBR322 and excised with *Bam*HI before heat-denaturation. The fragment *Eco*3-*Bam*2 contains introns *D–H*, the 3′ moiety of exon *4*(*4″*), exons *5–8* and the 5′ moiety of exon *9*(*9″*) (see line drawing and Fig. 4*c*), whereas fragment *Bam*3-*Bam*4 contains the 3′ moiety of intron *I*(*I″*), introns *J–P* and exons *10–17* (see line drawing and Fig. 4*d*). The two unnumbered arrows delimit the regions of the mRNA encoded by the 3′ moiety of exon *9* which is contained in the *Bam*2-*Bam*3 fragment (Fig. 4*a*, *b*) which was not present in this hybridisation. Length measurements are given in the legend to Fig. 4*c*, *d*. ***c*, Hybrids between con-mRNA and heat-denatured *Eco*3-*Bgl*II2 fragment of cloned *Eco*RI fragment 'a'.** The *Eco*3-*Bgl*II2 fragment (Fig. 4*a*) was prepared by digestion of the purified cloned *Eco*RI fragment 'a'. The hybrid regions contain the 3′ moiety of exon *4*(*4″*), exons *5–14* and introns *D–M* and the 5′ moiety of intron *N*(*N″*). (See line drawing and Fig. 4*e* for length measurements.) The unnumbered arrow points to the 5′ end of the RNA–DNA region which corresponds to the *Eco*3 site. ***d*, Hybrid between con-mRNA and the cloned conalbumin gene.** Hybrids were formed between con-mRNA and the heat-denatured complete conalbumin gene plasmid pBcon2 which was constructed from a modified pBR322 vector (containing a *Kpn*I site) by inserting the *Kpn*2-*Eco*3 fragment (Fig. 1*A*(*b*)) and the *Eco*RI fragment 'a' in the genomic order (M. Cochet, unpublished results). *Eco*RI fragment 'a' was purified from λWconA1, whereas the *Kpn*2-*Eco*3 fragment was obtained from λWconP5[1]. The hybrid region (2,508 ± 164 base pairs, 12 hybrid molecules were measured) contains all of the conalbumin exons *1–17* separated by the intronic loops *A–P* (see line drawing). In all cases the bars represents 0.1 μm.

These sequences were localised by restriction enzyme mapping using, as nick-translated probes, the two fragments and the total chicken DNA. Fragment *Eco* 'b' hybridises with both *Eco* 'c' (Fig. 6*A*, lane 1) and cellular DNA (Fig. 6*A*, lane 4) probes. The region which hybridises with both probes is localised in the *Pst* 'a' fragment [Fig. 6*A*, lanes 2 and 5, and Fig. 1*A*(*c*)]. However, the *Eco* 'c' probe hybridises only with the *Mbo* 'b' fragment [Figs 6*A*, lane 3; 1*A*(*c*)], whereas the cellular DNA probe hybridises strongly with fragment *Mbo* 'a' and only slightly to fragment *Mbo* 'b' [Fig. 6*A*, lane 6 and Fig. 1*A*(*c*)]. From these results we conclude that in a region located 2,000–3,200 base pairs upstream from the 5′ end of the conalbumin gene there is a highly repetitive sequence localised in fragment *Mbo* 'a' and a sequence situated in fragment *Mbo* 'b', which is repeated in *Eco* 'c', but not repeated very often in the rest of the genome. (We use the term highly repetitive, because these sequences must be repeated several hundreds of times in the genome for the total chicken DNA to be used as an efficient hybridisation probe in blotting experiments.)

Similarly, fragment *Eco* 'c' hybridises with *Eco* 'b' (Fig. 6*B*, lane 1) and cellular DNA (Fig. 6*B*, lane 4) probes. The region which hybridises with probes is localised in the *Pst* 'a' fragment of fragment *Eco* 'c' [Fig. 6*B* lanes 3 and 6, and Fig. 1*A*(*d*)], but the *Eco* 'b' probe hydridises only with the fragment *Sac* 'a' [Fig. 6*B*, lane 2, and Fig. 1*A*(*d*)], whereas the cellular DNA probe hydridises essentially with fragment *Sac* 'b' [Fig. 6*B*, lane 5, and Fig. 1*A*(*d*)]; on longer exposure exposure a faint hydridisation to fragment *Sac* 'a' was observed). From these results we conclude that the 3′ moiety of intron *C* [fragment *Sac* 'b', Fig. 1*A*(*d*)] contains a highly repetitive sequence, whereas the sequence which is similar to that present in the *Mbo* 'b' fragment of *Eco* 'b' is located in the 5′ moiety of intron *C* or in the 3′ moiety of intron *B* and is not repeated very often in the rest of the genome. Also, since the highly repetitive sequence present in *Eco*RI fragment 'b' is not revealed by *Eco* 'c' probe and conversely, our results demonstrate that the highly repetitive sequences contained in the conalbumin *Eco*RI fragments 'b' and 'c' belong to two different families of repetitive sequences.

It is interesting to compare our results with those recently reported by Eden and Hendrick[23], who found, by reassociation kinetic studies, that the repetitive chicken DNA can be divided into two classes based on repetition frequency: about one-third of the sequences are repeated 15-fold and two-thirds are repeated 1,500-fold. Also, they found that 40% of the chicken genome is arranged in an alternating pattern of single copy and repetitive sequences, with single copy regions of about 4,500 nucleotides in length, whereas almost one-half of the DNA consists of very long single copy regions, uninterrupted by repeated sequences for distance at least 17,500 base pairs. The two highly repeated sequences probably belong to the class of sequences which are repeated about 1,500-fold in the genome. It is striking that they are separated by 4,500 base pairs of single copy DNA as predicted from the reassociation kinetic studies. In contrast, their length is much shorter than that predicted by Eden and Hendrick[23] (at most 600 to 900 base pairs, instead of 2,000 base pairs). In contrast, there is no highly repeated sequence in the 10-kilobase pair-long conalbumin *Eco*RI fragment 'a' (L.M., unpublished result). In this respect, it is

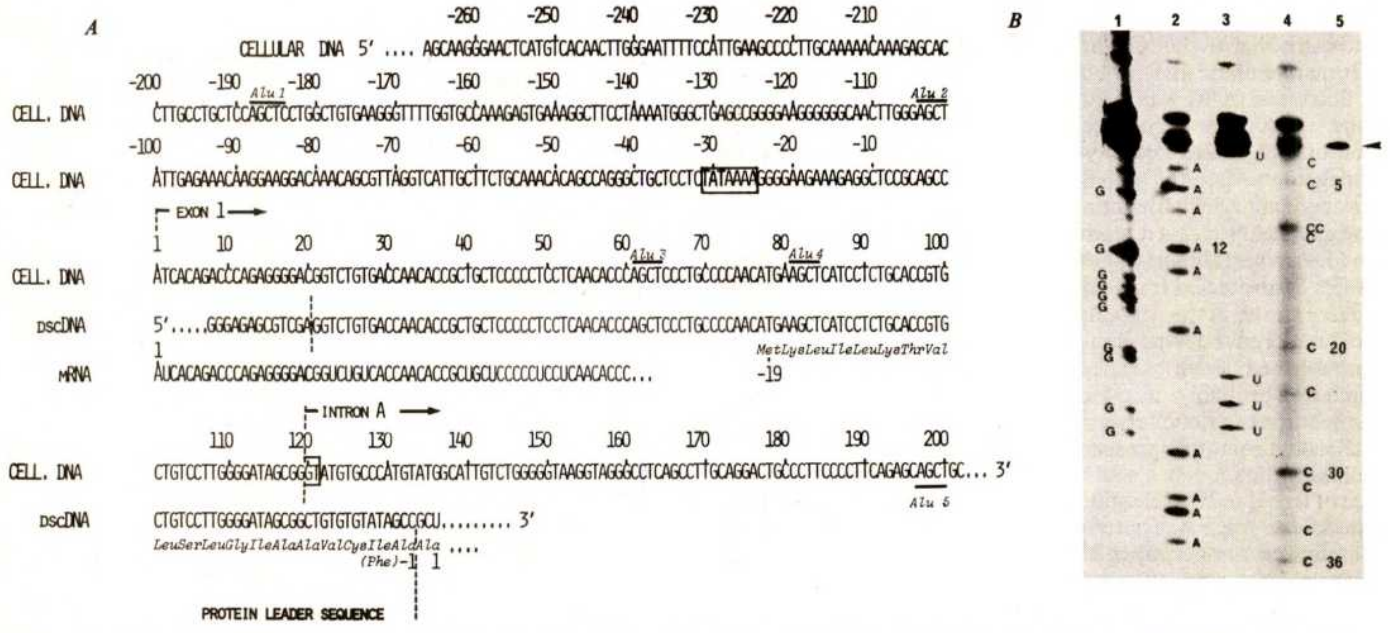


Fig. 5 ***A*, Comparison of nucleotide sequences at the 5′ ends of conalbumin double-stranded cDNA (dscDNA) and conalbumin mRNA (mRNA) with the corresponding sequences from genomic DNA (cell DNA).** The nucleotide sequences at the 5′ end of con-dscDNA and the genomic sequences within and around exon *1* were established as outlined in Fig. 1*B*. The first 60 nucleotides of con-mRNA were determined as described below and exemplified in *B* for the first 36 nucleotides. The DNA sequences (non-coding strand) were aligned with the mRNA sequence. The positive numbers refer to position of the nucleotides downstream from the 5′ end of mRNA, whereas negative numbers are given upstream from nucleotide number 1. The limit of exon *1* and of the 5′ end of intron *A* is shown in the genomic DNA sequences by a vertical dashed line. The dinucleotide GT[19] at the 5′ end of intron *1* is boxed. The positions of the *Alu*I sites (*Alu*1 to *Alu*5) shown in Fig. 1*B*(*c*) are indicated. The possible promoter AT-rich region (see text and Fig. 7*B*) is boxed. The limit separating the pBR322 sequence (TCGA corresponding to the *Taq*I site, see Fig. 1*B*(*b*) from the 5′ end of the dscDNA is indicated by a dashed line. The amino acid sequence of the protein leader sequence of pre-conalbumin[6] and the first amino acid of conalbumin are shown under the con-dscDNA sequence. A dashed line separates the protein leader sequence from the first amino acid (labelled 1) of the mature protein. The only amino acid difference (at position −2) between the sequence derived from the dscDNA and that obtained by amino acid sequencing[6] is indicated. ***B*, Autoradiogram of sequencing gel showing the sequence at the 5′ end of con-mRNA.** The sequence was obtained by the chain termination method of Sanger *et al.*[10] as modified by Akusjärvi and Petterson[20] in order to sequence reverse transcripts of mRNA. An *Alu*I digest of 0.3 μg of the genomic *Pst*5-*Pst*6 fragment (see Fig. 1*B*(*c*)) was denatured and hybridised with 60 μg of total hen RNA (purified on oligo dT-cellulose) as described in ref. 20, but for 1 h at 37 °C. The chain elongation (using [α-^{32}P]-labelled TTP) and the chase step were carried out at 37 °C as in ref. 20, but reverse transcriptase was added again before the chase step which was prolonged for 16 hours. Lanes 1–4 correspond to the reaction mixtures containing the dideoxynucleotides ddCTP, ddTTP, ddATP and ddGTP, respectively (the bases indicated in the figure correspond to those of the mRNA and are numbered from its 5′ end). The position corresponding to the first nucleotide of the mRNA was determined as follows: an *Alu*3-*Alu*4 fragment labelled at its 5′ ends with [γ-^{32}P]ATP and T4 polynucleotide kinase was purified by electrophoresis on a 8% acrylamide gel, hybridised to oligodT-cellulose purified hen RNA as above, and incubated with reverse transcriptase and the four deoxynucleoside triphosphates for 2 h at 37 °C. The elongated primer was run in lane 5 concomitantly with the sequencing reactions run in lanes 1 to 4, and after autoradiography yielded a single band (arrow head) indicating that the first nucleotide of the mRNA is the one just above the first U. The bands, which in lanes 1–4 are situated above the arrow head, correspond to elongation products of *Alu*1-*Alu*2 and *Pst*5-*Alu*1 fragments (see Fig. 1*B*(*c*)) which were present in the almost (but not fully) complete *Alu*I digest of the *Pst*5-*Pst*6 fragment used to prime in the reactions run in lanes 1 to 4 (see above). (These bands were not seen when purified *Alu*3-*Alu*4 fragment was used as a primer.)

interesting to recall the absence of repetitive sequences for more than 17 kilobase pairs within and around the ovalbumin gene[9,16,24]. The repetitiveness of the sequence which is common to *Eco*RI fragments 'b' and 'c' is unknown and the sequence might belong to the class of 15-fold repetitive sequences. It is interesting that in both cases they are situated close to highly repeated sequences. Current DNA sequence studies are further analysing these two classes of repeated sequences in an attempt to elucidate their relationship, if any.

Possible control regions at the 5′ end of conalbumin mRNA and around the 5′ end of the conalbumin gene

As for most eukaryotic mRNAs which have been sequenced up to now[25], the AUG codon located closest to the 5′ end of con-mRNA is the one used to initiate translation. Although the mechanism for translation-initiation site selection in eukaryotes is still unknown[25], the striking conservation of the 3′-terminal sequence of 18S RNA among eukaryotes led to the suggestion that this region of the 18S rRNA might base-pair with the complementary sequence of the initiation region in mRNA[26]. Complementarity between the 18S rRNA sequence, 3′-UAGGAAGGCGU-5′ and sequences of the 5′-non-coding regions of many eukaryotic mRNAs (see refs 25, 26 and refs therein), including ovalbumin mRNA[24,27] (Fig. 7*B*), has been recognised. In the case of conalbumin, there are two such possible regions of complementarity (Fig. 7*A*), although both are shorter than for ovalbumin mRNA. It is striking that, like ovalbumin, one of these regions is immediately preceded by a sequence which can form a base-paired loop (nucleotides 2–29). Although the existence of a 5′-terminal loop is not a general feature of eukaryotic mRNA, there is a similar hairpin structure at the 5′ end of SV40 VP1 mRNA[28]. The possible role of such hairpin structures in the capping and/or in the translation-initiation processes remains to be established by site-directed mutagenesis.

A putative AT-rich promoter region (Hogness box) for RNA polymerase B (or II) has recently been proposed on the basis of a comparison of DNA sequences from several genes and is located 23–25 base pairs upstream from the base coding for the first nucleotide of the mRNA (see refs 24, 29 and refs therein). Similarly, a possible promoter site is also present 25 base pairs upstream from the conalbumin cap site (Fig. 7*B*). When compared to other putative promoter sites[24], it is apparent that in most cases the AT-rich region is flanked on both sides by GC-rich segments, indicating that the final length of this possible eukaryotic promoter sequence will probably be very similar to that of the extended Pribnow box[30]. There is an amazing identity extending over 12 base pairs between the promoter sequence of the conalbumin gene and that of the major late adenovirus genes. In fact, this striking similarity is not limited to the Hogness box, but two additional regions of identity, 5 and 9 base pairs long, are found at positions −67 to −71 and −83 to −91, respectively. All of these identities, both in sequence and in position with respect to the cap site, cannot have occurred by chance and must correspond to common functional sites shared by these two evolutionarily distant genes. Again, site-directed mutagenesis studies will be required to investigate whether the regions of identity located upstream from the Hogness box could correspond to a site similar to the prokaryotic promoter recognition site[31]. In this respect, it is interesting that there are some similarities between the sequences present in these regions of the conalbumin and adenovirus late genes and sequences present in the corresponding regions of ovalbumin and other genes[32].

On the other hand, although there are some additional similarities in the region corresponding to the first 250 base pairs upstream from the cap site when comparing the ovalbumin and conalbumin gene sequences[24,32], they are, surprisingly, much less impressive than those noted above for the conalbumin and adenovirus late major genes. This result could indicate that there is no steroid hormone-mediated recognition of the DNA sequences present in this region. Alternatively, it may reflect the known difference of the ovalbumin and conalbumin genes in their hormonal response. Forthcoming sequence data for genes such as those for ovomucoid and lysozyme, and genes *X* and *Y* (refs 33, 34) whose hormone response is more like ovalbumin should be helpful in deciding between these two possibilities. Finally, although there is a striking similarity between the 10 residues which precede the cap site of adenovirus-2 late major and mouse β globin major genes[35,36], this homology sequence does not seem to be a general feature for eukaryotic messenger-coding genes, since it is absent from the conalbumin gene just as it is from other genes[24].

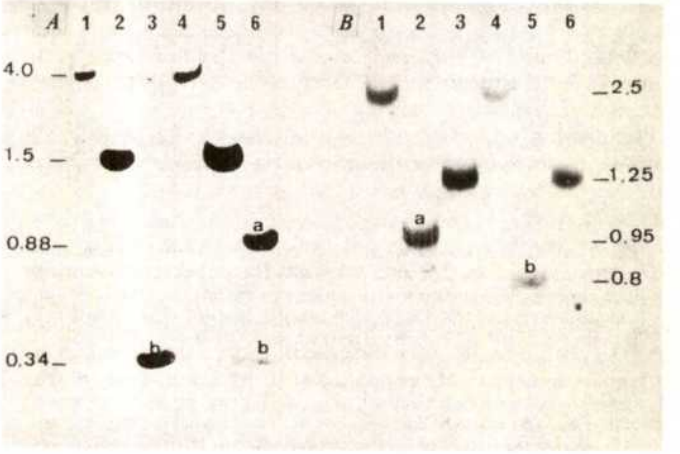


Fig. 6 Presence of repetitive sequences in the conalbumin *Eco*RI fragments 'b' and 'c'. *A*, Hybridisation of *Eco*RI fragment 'b' (lanes 1 and 4), and its *Pst*I (lanes 2 and 5) or *Mbo*II (lanes 3 and 6) digests with *Eco*RI 'c' fragment (lanes 1–3) or total cellular chicken DNA (lanes 4–6) labelled with ^{32}P by nick-translation. *B*, Hybridisation of *Eco*RI fragment 'c' (lanes 1 and 4) and its *Sac*I (lanes 2 and 5) or *Pst*I (lanes 3 and 6) digests with *Eco*RI 'b' fragment (lanes 1–3) or total cellular chicken DNA (lanes 4–6) labelled with ^{32}P by nick-translation. The intact *Eco*RI 'b' (0.2 μg) or *Eco*RI 'c' (0.2 μg) fragments and their restriction enzyme digests were electrophoresed on a 2% agarose slab gel, transferred to nitrocellulose filters, hybridised with the labelled probes and revealed by autoradiography as described in legend to Fig. 2. Exposure time for autoradiography was 3 days for lanes 1–6 of panel *A*, 6 days for lanes 1–3 of panel *B* and 1 day for lanes 4–6 of panel *B*. a And b refer to hybridising fragments [see text and Fig. 1*A*(*c*), (*d*)].

Evolutionary and functional aspects of the organisation of the conalbumin gene

The conalbumin gene represents the most striking example of a split gene described to date. It is remarkable that, like the ovalbumin[19,24], ovomucoid[37,38], lysozyme[39,40] and *X* and *Y*[33,34] genes, all of the conalbumin exons are short with sizes ranging from 50 to 200 base pairs. Any attempt to explain the emergence and evolution of split genes should take into consideration the small size and the high number of exons in these genes. In particular, it seems difficult to accept the idea (see ref. 41 and refs therein) that these genes have evolved from already distinct exons, each coding for a different structural domain, and that they were brought together in the genome by random shuffling. It is noteworthy that, although con-mRNA is only 25% longer than ovalbumin mRNA, the mRNA-coding portion of the conalbumin gene is in 17 pieces, whereas the ovalbumin gene is in eight pieces. In this respect the conalbumin gene is more like the ovomucoïd gene[37,38] which possesses at least seven exons for an mRNA of about 800 nucleotides. We have learnt from the study of the ovalbumin and the ovalbumin-related *X* and *Y* genes[33,34] that new split genes could have evolved by duplication of an ancestral gene which was already split in the same way. A similar conclusion was reached by analysing the structure of the α and β globin genes[42]. Such an origin of the conalbumin gene is

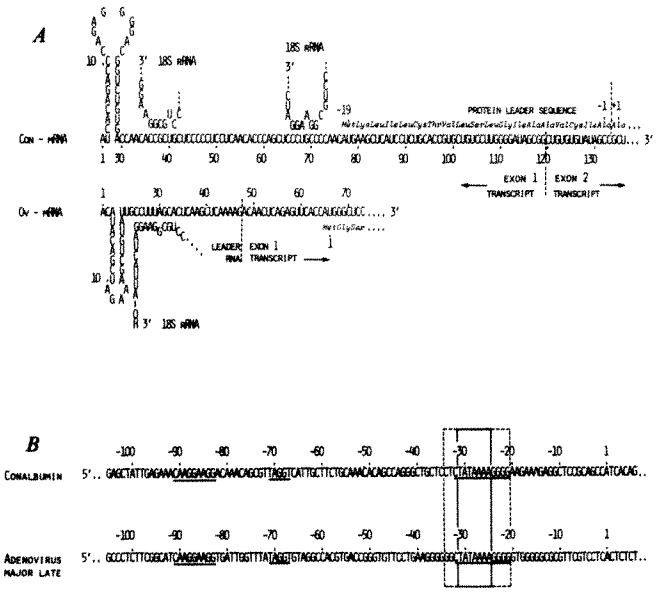


Fig. 7 *A*, Comparison between the 5′ end region of conalbumin (con-mRNA) and ovalbumin (ov-mRNA) mRNA. The con-mRNA sequence was taken from Fig. 5*A* and the ovalbumin sequence from Fig. 5 of ref. 24. The complementarity of con-mRNA with two sequences present at the 3′ end region of 18S rRNA is shown (see text). *B*, Comparison between DNA sequences (non-coding strands) of conalbumin and adenovirus-2 major late genes upstream from the regions coding for the 5′ end of the mRNAs (position 1). The AT-rich and GC regions of homology are boxed with a continuous and a dashed line, respectively. The lines below the sequences indicate the regions of identity between the two DNA sequences.

unlikely, since we found no evidence for sequences cross-hybridising with the conalbumin exonic DNA in the chicken genome. However, the determination of the amino acid sequence of human and of chicken transferrins has provided very strong evidence that doubling of an ancestral structural gene occurred during the evolution of transferrins (refs 43–45 and J. Williams, personal communication). It therefore appears more likely that the present day conalbumin gene has evolved by duplication of an already split ancestor gene (perhaps in seven or eight pieces, like the present day ovomucoïd gene). In this respect, the mechanism responsible for the appearance of the present day conalbumin gene could be similar to the duplication-fusion mechanism recently proposed by Sakano *et al.*[46] for the origin of the constant region of the mouse immunoglobulin γ1 heavy chain, except that in the conalbumin case the ancestral gene would already be a split gene. Clearly, the current detailed studies of the conalbumin gene structure should be very informative upon the question of how a new split gene may evolve by duplication of an ancestral split gene—a fundamental question, since gene duplication has perhaps been the predominant force involved in evolution[47].

It has been suggested that each exon might correspond to a functional or a structural domain (see ref. 41 and refs therein). Conalbumin provides an opportunity to test this idea, since the first 19 amino acids of pro-conalbumin belong to a functional domain which is involved in the secretion of the protein[6,48]. Although it is not known which amino acids are absolutely required for the secretion of the protein, 14 out of the 19 amino acids which constitute the protein leader sequence are encoded in exon *1* (Figs 5*A*, 7*A*). A similar situation is encountered in the case of the mouse immunoglobulin light chain genes where 15 out of the 19 amino acids which constitute the protein leader sequence are encoded in exon *1*[49,50]. It remains to be seen whether this correspondence between exon *1* and the possibly functional portion of the protein leader sequence is coincidental or reflects a general feature of the organisation of genes coding for secreted proteins possessing a protein leader sequence. The existence of two homologous iron-binding domains in transferrins[43–45,51] will provide an additional possibility to test the idea that exons could correspond to structural or functional domains.

Finally, the presence of repetitive sequences in intron *C* of the conalbumin gene is interesting in at least two respects. Firstly because, if the present day conalbumin gene has evolved by duplication of an ancestral split gene which was in seven or eight pieces, it is surprising that no repetitive sequences are found in the conalbumin *Eco*RI fragment 'a'. This absence might reflect the insertion of the repetitive sequences at a later stage during evolution or the loss of the duplicated counterpart. Secondly, the presence of these repetitive sequences should allow us to answer some questions related to their possible structural and/or regulatory role[52].

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Hepatitis B virus genes and their expression in *E. coli*

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A composite DNA sequence of regions of hepatitis B virus, determined from a series of recombinant plasmids, reveals the genes for the surface antigen and the core antigen of the virus. The sequence of the core antigen shows it to be a DNA binding protein. The core antigen gene is expressed in Escherichia coli *and when injected into rabbits the bacterial product induces antibodies which react with core antigen isolated from human sources.*

DNA FROM hepatitis B virus (HBV) of three different serotypes has been cloned in *Escherichia coli* via phage λ derivatives[1,2] and plasmids[3,4]. Some of the transformed cells produce an antigen that reacts specifically with hepatitis B core antibodies (HBcAb)[3]; these cells contain hybrid plasmids, constructed by insertion of HBV DNA fragments into pBR322 at the R.*Pst*I cleavage site via 3′ oligo (dG) and oligo (dC) tails to facilitate the expression of viral antigen genes as polypeptides fused to β-lactamase[5]. Thus, the coding sequence of the HBV core antigen should be adjacent to or near the point of fusion to the β-lactamase gene. Nucleotide sequence analysis might identify HBV genes since the molecular weights of the core antigen (HBcAg) and surface antigen (HBsAg) are known[6,7], as are partial amino acid sequences from the N- and C-termini of HBsAg[8].

DNA fragment mapping and nucleotide sequence determination

The cloning of HBV DNA fragments isolated from blood plasma with the complex serotype *adyw* was described previously[3], together with some properties of some of the recombinant plasmids. The viral DNA has an unusual structure: there is a nick in one strand and a gap in the other[6]. Table 1 lists the plasmids chosen for sequence analysis and shows the sizes of the HBV fragments that they contain. All those plasmids imparting to their host cell the ability to synthesise core antigen, as detected in the solid phase radioimmunoassay[9], contained HBV sequences in the same orientation; in some cases this was confirmed by heteroduplex electron microscopy. Fragments were prepared from these plasmids by digestion with appropriate restriction enzymes and labelled at their 5′ termini with [γ-^{32}P]ATP and T4 polynucleotide kinase, and their nucleotide sequences were determined by the chemical degradative methods of Maxam and Gilbert[10]. Figure 1 outlines the sequencing strategy and Fig. 2 shows the composite sequence. The sequence shown covers about 87% of the virus; our set of clones has proved deficient in representations of the region of the gap in the viral DNA.

Table 1 HBV clones

HBcAg positive

Clones	Ala_{182}	Left endpoint	Phe	His	Right endpoint
pHBV64	GCA	$(GGG)_5GTT$	TTT	CAC	1,788
pHBV66	GCA	$(GGG)_6GAA$ CTT	TTT	CAC	~1,710
pHBV139A	GCA	$(GGG)_8GGA$ CTT	TTT	CAC	~1,260
pHBV110	GCA	$(GGG)_7CTT$	TTT	CAC	~1,240
	GCA	$(GGG)_6GGC$	TTT	TTC	
pHBV138	GCA	$(GGG)_5CTT$	TTT	CAC	1,842
pHBV139	GCA	$(GGG)_7CTT$	TTT	CAC	1,625

HBcAg negative

Clones	Left endpoint	Right endpoint
pHBV15	1,967	2,656
pHBV20	1,447	2,199
pHBV114	~−80	~2,270

The structure of the oligo (dG) stretch that fuses to Ala_{182} of β-lactamase is given for the HBcAg producing plasmids. Clone pHBV110 has two plasmids, one out of phase by one base.

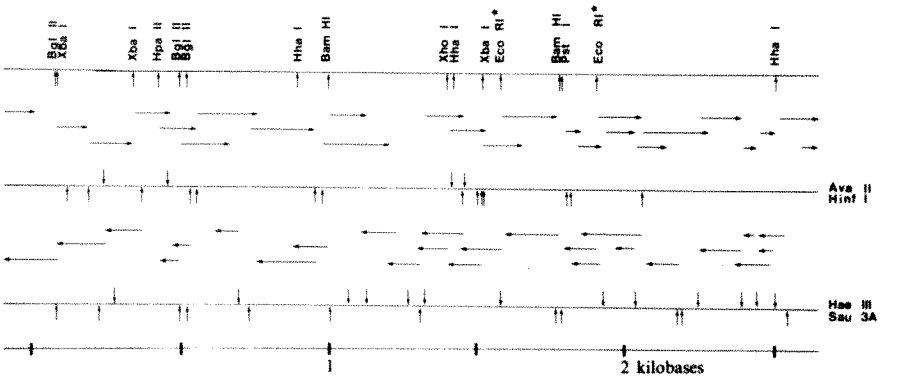


Fig. 1 The positions of restriction targets in HBV DNA and the strategy for nucleotide sequence determination. For simplicity the restriction targets are distributed between three maps, but each covers the same DNA sequence which, like the sequence shown in Fig. 2, begins at the point of attachment to the *Pst* site in pBR 322. Vertical arrows indicate the positions of restriction targets and the horizontal arrows show the direction and extent of the sequencing runs; the sequencing techniques were the chemical methods of Maxam and Gilbert[10].

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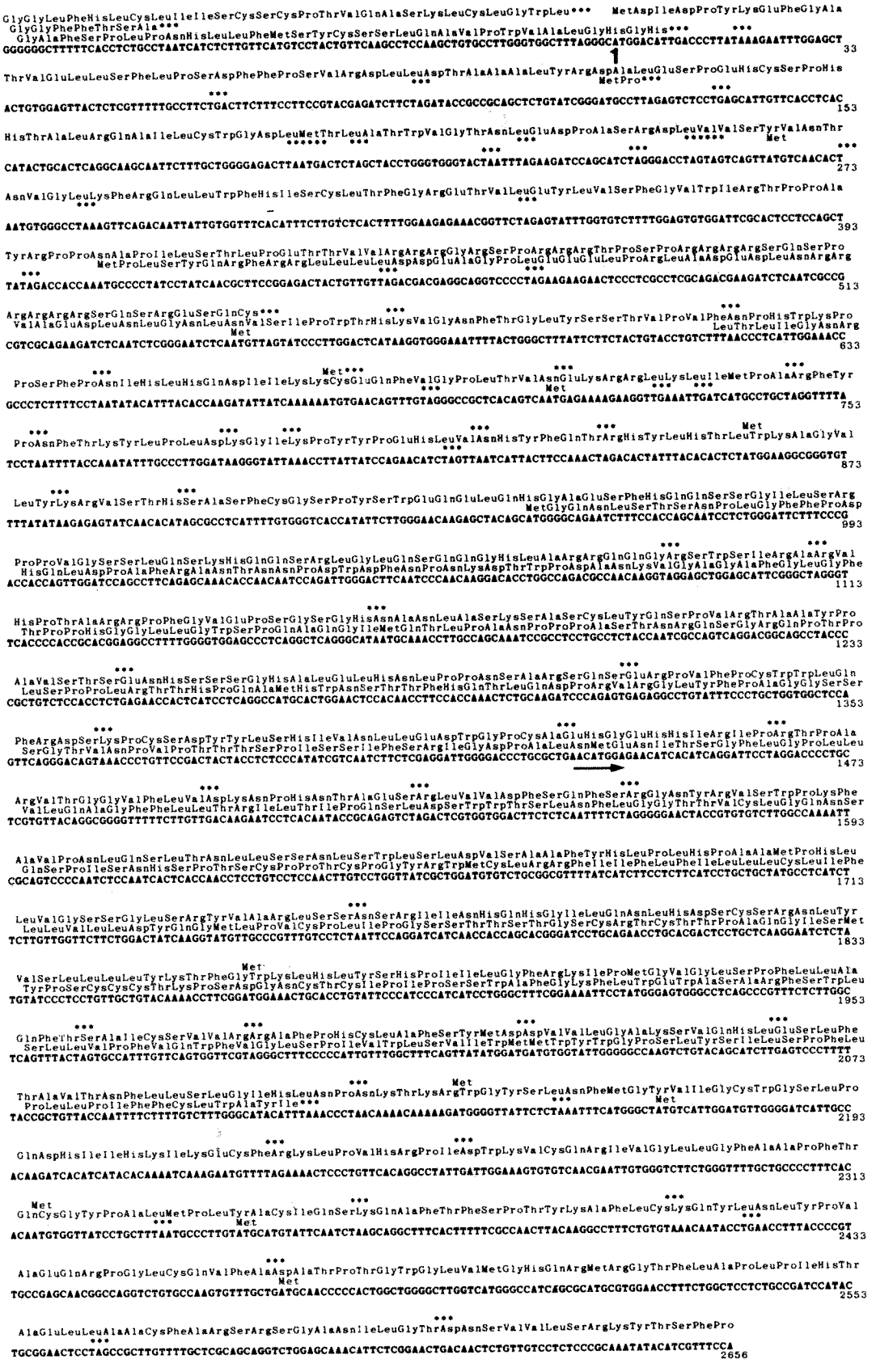


Fig. 2 The nucleotide sequence of HBV DNA shown as fused into pBR322 and expressing HBcAg (the first phase). The major possible translation products in all three reading frames are shown starting at the first ATG after a terminator***. The sequence is numbered from the A of the ATG for initiation of the core antigen as reference. Core antigen sequence runs from residues 1 to 549; the surface antigen is encoded between residues 1,437 and 2,114. The sequence was derived principally from clone pHBV138 and was extended by results derived from pHBV20 and pHBV15. The arrow shows the beginning of the sequence coding for HBV surface antigen. Several shorter translation products are possible, but have been omitted from the figure for simplicity.

The core antigen gene

All of the recombinants examined that produced HBcAg proved to have the HBV DNA insertion in the same phase with respect to the R.*Pst*I site in the β-lactamase gene beginning within one or two bases of the same sequence. Table 1 lists the structure of these junctures. Since the original HBV fragments came from digests with different restriction enzymes, R.*Bam*HI and R.*Kpn*I, this unique attachment was surprising. It may have resulted from inadvertant breakage, or tailing, of the DNA (from Dane particles) at the nick in the molecule[6]. We expected that the core antigen, in whole or in part, would be fused to β-lactamase via a small number of glycine residues, but that is not the case. In six strains that produce antigen, the β-lactamase is fused through 5 to 8 glycines to the same peptide sequence, but this sequence terminates 25 amino acids further on. In this phase the stop codon is followed three nucleotides later by an initiation sequence from which translation continues unhindered to give a polypeptide of 183 residues (Fig. 2, positions 1 to 549). Stop codons are abundant in the other two translation phases throughout this region.

Because there is a requirement for in-phase reading of this termination signal, the situation resembles the restart phenomenon observed in bacterial proteins. We conclude that the core antigenic activity resides in a polypeptide of molecular weight 21,000 translated *de novo* from the transcript of the viral DNA sequence. This product would probably remain within the cell, rather than be within the periplasmic space; this is consistent with our observations that HBcAg activity is only detected in bacterial extracts, not in osmotic 'shockates'.

The core antigen

Amino acid sequences for the *in vivo* core antigen have not been published, but the calculated size of the antigen resulting from translation of the nucleotide sequence carried in the bacterial plasmid is close to the molecular weight of 19,000 observed for the antigen isolated from Dane particles[6,7]. We do not know if any amino acids have been trimmed from the amino terminus of the authentic protein. The deduced polypeptide sequence is interesting; while the protein is not particularly remarkable in its amino-terminal half, sequences homologous with protamines and especially with galline[11] (from rooster sperm) are found towards the carboxy terminus (residues 150–183, nucleotides 448–549). In this region 16 out of 34 amino acids are arginines, arranged in groups of 3 or 4 separated by 4 other residues (Fig. 2). The sequence Ser-Pro-$(Arg)_3$ occurs three times, while there is a double repeat of the sequence Ser-Pro-$(Arg)_4$-Ser-Gln. The obvious inference is that the C-terminal region binds the HBV DNA within the Dane particles, while the rest of the protein participates in other structural roles. The four cysteine residues offer the possibility of internal disulphide bridges as well as intermolecular interactions, the latter possibly explaining some of the higher molecular weight values observed for HBcAg[6].

Although the structure of the messenger for HBcAg is unknown, we support the inference that the ATG at position one is the initiator by noting a secondary structure feature in the DNA sequence, and thus in a hypothetical mRNA, just before that ATG: a stem with a loop that could pair with the 18S ribosomal RNA analogous to structures that have been found in preproinsulin messages and some other higher cell mRNAs[12–14]. Figure 3 shows this feature. Although short oligonucleotide sequences that would possess a similar capacity for such interactions can be found elsewhere in the HBV DNA sequence, none can be related to an initiation codon in a simple way.

In vivo activity of the core antigen produced from *E. coli*

To test the biological activity of the antigenic polypeptide translated from the plasmid transcript, sterile extracts of the bacterial cells mixed with an equal volume of Freund's adjuvant were injected into rabbits. Two animals received the crude bacterial extract, and two were given samples of the same extract after fractionation on a Sephadex G-50 column. Additional injections of the same sample, which had been stored frozen and shown to retain full antigenic activity, were given 2 weeks and 5 weeks after the first injection, and the animals were bled at intervals several weeks after the initial injection. Figure 4 shows the results of immunodiffusion experiments[15] comparing these sera to human HBcAb (HBcAg positive), using HBcAg derived from human liver[16]. All four rabbit sera gave precipitin lines with HBcAg identical to those formed between this antigen and HBcAb derived from human sources. Thus, the core antigen synthesised in *E. coli* is serologically active *in vivo*. Regardless of whether antibodies to this particular antigen confer protection against HBV *in vivo*, the experiment does establish the feasibility of vaccine production from viral antigens synthesised in microbial cells.

Table 2 Amino acid composition for HBsAg

Serotype:	adw*	ayw3*	ayw†	adyw‡
Total residues:	200	200	220	226
Ala	7	10.2	7	6
Asp	11.2	13.4	11	3
Asn	—	—	—	5
Arg	4.4	6.0	7	6
Cys	14.4	10.2	—	14
Glu	11.6	15.2	10	2
Gln	—	—	—	7
Gly	12.1	18.4	19	15
His	1.4	1.0	1	1
Ile	11.4	10.8	11	17
Leu	22.4	30.8	36	35
Lys	4.4	3.8	3	2
Met	6	6.2	6	6
Phe	10	12.6	15	16
Pro	25	24.8	26	21
Ser	23.8	28.0	25	26
Tyr	4.8	5.2	5	6
Thr	16.8	21.0	21	16
Trp	—	—	—	13
Val	8.2	10.6	11	9

* From Peterson *et al.*[21].
† D. L. Peterson, personal communication.
‡ This work.

The surface antigen gene

None of the hybrid plasmids used in the sequence analysis synthesised any surface antigen that could be detected by the radioimmunoassay. However, amino acid sequences of parts of HBsAg are known[8] so that a corresponding gene sequence could be sought along the DNA. Figure 2 shows such a sequence, starting at residue 1,437, which runs from the observed N

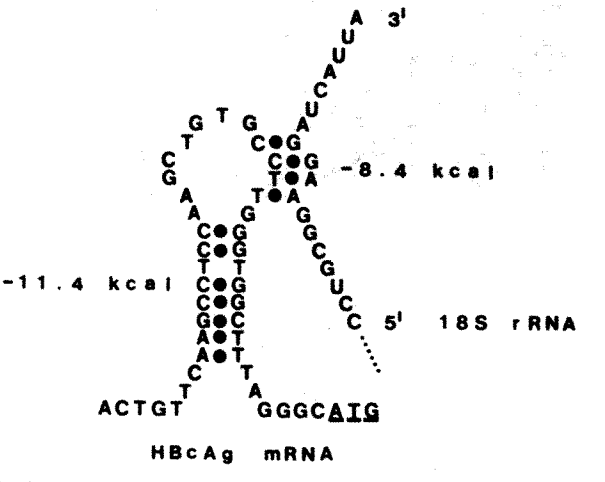


Fig. 3 A secondary structure feature lying immediately before the initiator of the core antigen which could pair with the 18S rRNA.

Table 3 Hepatitis B sequence heterogeneity

Gene/position of initial nucleotide	pHBV138	pHBV64	pHBV139	pHBV110	pHBV20	pHBV170
HBcAg/376	ATT Ile					GTT Val
HBcAg/424	ACG Thr	ACA Thr				
(pre?)HBsAg/1,236	CTG Leu			GTG Val		
(pre?)HBsAg/1,431	CTG Leu	CGG Arg				
HBsAg/1,575	GTG Val	GTG Val	GCG Ala		GTG Val	
HBsAg/1,623	ACC Thr		AGG Arg		ACC Thr	
HBsAg/1,764	ATT Ile				CTT Leu	

terminus, Met-Glu-Asn-Ile-Thr-Ser- (ref. 8) to a terminator 226 amino acid residues away. We presume that this protein is initiated at the ATG at residue 1,437, but we cannot exclude the possibility that the mature protein is cleaved from a larger polypeptide initiated at the ATG at residue 948, since there is no termination codon between these two triplets. This polypeptide sequence, corresponding to a protein of molecular weight 25,400, also contains two peptide sequences of nearly identical composition to those for two amino-ethyl-cysteinyl peptides of 13 and 28 amino acids in length isolated after digestion of HBsAg with trypsin (D. L. Peterson, personal communication), as well as the correct C-terminal dipeptide[8], all in the same reading frame. The amino acid composition of HBsAg deduced from the nucleotide sequence is in accord with the results of analysis of the antigen itself (Table 2). The molecular weight of HBsAg in the glycosylated form has been estimated to be 25,000, while that of the nonglycosylated form of this polypeptide, which is also immunogenic, has been estimated to be 22,000[7,8]. There is one characteristic glycosylation sequence in this protein, the Asn in the third position, which lies in a . . . Asn-Ile-Thr-Ser . . . sequence[17]. The location in the cloned HBV DNA of a nucleotide sequence corresponding with amino acid sequences of HBsAg is strong evidence that the surface antigen is a viral gene product and not a host gene product induced by infection with the virus. The donor of the plasma used as a source of Dane particles for these clonings showed a complex serotype, a mixture of *adw* and *ayw*, and presumably was infected by two viruses. We have observed microheterogeneity between different clones. Table 3 lists these sequence variations (not exhaustively). Several of the variations are silent, or not in known products, but three changes occur in HBsAg, substitution of Ala for Val and Arg for Thr between clones pHBV138 and pHBV139 as well as a change of a Leu for Ile in a third clone, and these could reflect this complex serotype. The sequence heterogeneity of the different clones demonstrates that the infection involves a heterogeneous virus population. Furthermore, the gene for surface antigen extends into the region of the gap in the HBV DNA molecule and may be drifting rapidly.

The nucleotide sequence of the recombinant plasmids shows that none of those examined has the HBsAg gene in a position from which we would expect any biologically active polypeptide to be produced, and no HBsAg was detected. The sequence results immediately indicate subcloning experiments for the expression of the surface antigen as a polypeptide fused to β-lactamase. Some of the clones obtained from these experiments have given positive reactions in the radioimmunoassay for HBsAg.

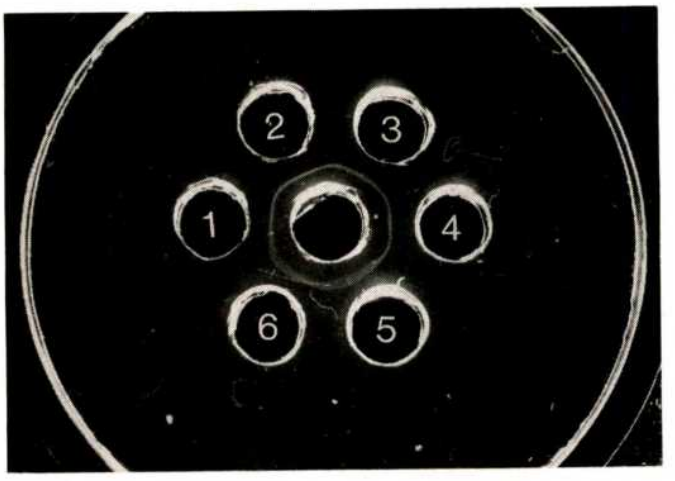


Fig. 4 An Ouchterlony immunodiffusion experiment comparing rabbit anti-(*E. coli*-HBcAg) to human anti-HBcAg in a 0.9% agarose gel in 0.01 M Tris-HCl *p*H 7.2, 0.001 M EDTA, 0.1M NaCl. The centre well contained partially purified HBcAg derived from human liver[16]. Wells 1 and 4 contained human HBcAb at a dilution of 1 in 30, wells 2 and 3 contained undiluted serum from rabbits injected with the unfractionated extract from bacteria carrying clone pHBV139, and wells 5 and 6 contained undiluted rabbit antiserum raised against HBcAg from the same bacterial extract, but fractionated on a column of Sephadex G-50. Equivalent results were also obtained in a solid phase radioimmunoassay.

Other HBV genes

In addition to the core and surface antigens, the *e* antigen (HBeAg) and the endogenous DNA polymerase of the Dane particle should be encoded in the viral DNA; however, little is known of their properties. The DNA sequence suggests that the viral information is encoded in overlapping readings in one direction. The largest translation produce encoded anywhere by the opposite DNA strand is 62 amino acids, while if the HBV nucleotide sequence is read in the third reading frame (with respect to core and surface antigen sequences), a translation product overlapping the C-terminal region of the core antigen as well as the entire gene for surface antigen continues for at least 750 amino acid residues (to nucleotide 2656 which is the extent of the sequence so far determined) and so could perhaps be a candidate for the DNA polymerase. Bacterial and viral DNA polymerases have molecular weight in the range 109,000–120,000[18,19], whereas that of the *e* antigen is probably around 19,000[20].

In this discussion we have assumed for simplicity that there is no important splicing of the viral RNA within the translated regions of the genome. However, a full understanding of the mechanism of expression of the viral genes must await an identification of the structure of the viral messages. Now that cloned fragments and DNA sequences of HBV are available, a complete unravelling of the molecular biology of this human disease is possible.

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Equilibrium: the intracellular distribution of steroid receptors

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Data are presented which suggest that there are unbound receptors for oestrogen in nuclei of the smooth muscle cells of the myometrium. A new model for the distribution of unbound receptors is proposed in which unbound receptor is in equilibrium, partitioned between nucleus and cytoplasm according to the free water content of these intracellular compartments.

THE most widely accepted model for the intracellular translocation of steroids was developed from many studies concerning the uptake and retention of oestradiol in cells of the female reproductive tract. According to this model, oestradiol enters the uterine cells, binds to a specific high-affinity cytoplasmic receptor and is then translocated to the nucleus by a temperature sensitive process. During this process, the oestrogen receptor undergoes a conformational change which is discernible by an increase in its sedimentation coefficient from 3.8–4S to 5–5.2S and by its ability to bind to isolated uterine nuclei and stimulate RNA synthesis[1–3]. However, there are data in the literature which question the validity of this model (for review see ref. 4). Briefly, free receptor and 4S receptor have been found in carefully washed nuclei[5–9], and the conversion of 4S to 5S has been shown to take place more rapidly in the presence of DNA[10]. Much of the support for the classical model for the intracellular translocation of steroids comes from autoradiographic studies conducted together with the early biochemical studies[11]. After a 1-h incubation at 37 °C the steroid was localised autoradiographically in the nucleus, agreeing with the biochemical data, whereas after a 5-min incubation at 2 °C the steroid was localised autoradiographically outside the nucleus. Close examination of the autoradiogram shown[11] does not reveal a cytosol concentration of steroid but rather a diffuse pattern resembling background. This interpretation does not agree with the corresponding biochemical data[11]. Because we were unconvinced by the autoradiogram and because in our hands whole uteri are subject to problems associated with diffusion and washing, we initiated experiments which we feel demonstrate unbound nuclear receptors in rat uterus and a breast tumour line. In addition, we propose a new model for the intracellular distribution of steroid receptors in which unbound receptor is in equilibrium, partitioned between cytoplasm and nucleus according to their respective free water content.

Autoradiographic studies: nuclear receptor at 4 °C

The immature rat uterus was studied in detail because it was used by the original investigators to establish the current model for the mechanism of action of steroids. A second system was used to confirm these results—cells from a breast tumour line designated MCF-7.

Whole uteri from 21–23-d-old rats (Sprague–Dawley) were incubated in minimal essential medium (MEM, GIBCO) in the presence of 5×10^{-9}M [1, 2, 6, 7-^{3}H]oestradiol-17β (89 Ci mmol^{-1}, Amersham/Searle) with or without 5×10^{-7} M unlabelled oestradiol (E_2, Sigma) for either 5 min or 2 h at either 0 or 37 °C. Identical incubations were carried out with ^{3}H-R2858([6,7-^{3}H]methoxy-17 ethyloestradiol, 52 Ci mmol^{-1}) or with ^{3}H-R2858 + unlabelled R2858. The tissue was then processed for autoradiography[12] to determine the location of the ^{3}H-steroid. Additionally, some tissues were incubated for 5 min at 0 °C and processed biochemically (see below). Nuclear local-

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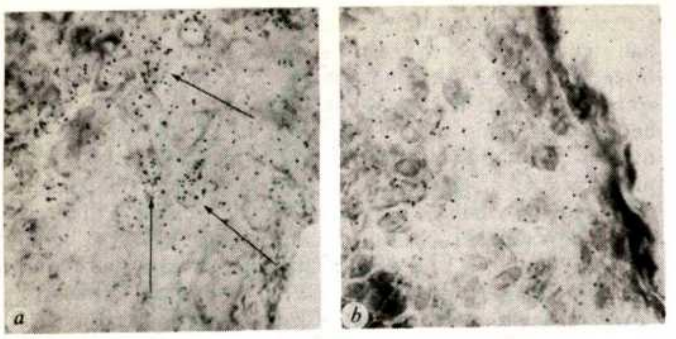


Fig. 1 Autoradiograms of rat uteri from the extreme periphery of the sections. The serosa appears to the right in both autoradiograms. *a*, Incubated at 0 °C for 5 min with ^{3}H-oestradiol-17β (5×10^{-9}M), *b*, incubated at 0 °C with ^{3}H-oestradiol-17β (5×10^{-9}M) + unlabelled oestradiol-17β (5×10^{-7}M). Note the nuclear localisation (arrows) of silver grains over myometrial cell in *a* and the lack of grains over nuclei in *b*. Stained with methylgreen-pyronin, 40-d exposure, 4-μm section, 500×.

isation was found at 0 °C at both 5 min (Fig. 1) and 2 h (not shown) in the myometrium at the extreme periphery of the section. Autoradiograms from uteri incubated at 0 °C indicated that there was little diffusion of the ^{3}H-steroid even after the 2-h incubation (Fig. 2), and thus the inner portion of the myometrium and the endometrium were unlabelled. Also at neither time was there any indication of an exclusive cytoplasmic localisation of ^{3}H-steroid. Nuclear localisation was also found at 37 °C at 5 min and 2 h (not shown). We repeated these experiments three times with ^{3}H-oestradiol and three times with ^{3}H-R2858 (Fig. 3) and obtained identical results.

Our autoradiographic localisation of a nuclear concentration of ^{3}H-E_2 and ^{3}H-R2858 after 5 min of incubation at 0 °C is in direct conflict with the earlier autoradiographic results[11]. The reason(s) the earlier workers did not see nuclear localisation is (are) unclear. Possible explanations are the differences in specific activities (earlier work 57.4 cm mmol^{-1} compared with our 89 Ci mmol^{-1}) and exposure time (in the earlier work localisation was found in 45 d with dry-mount technique while in our work the earliest localisation was found at 30 d with the thaw-mount technique, which is usually two to three times faster than the dry-mount technique).

MCF-7 cells were also incubated in MEM in the presence of different concentrations of ^{3}H-E_2 or ^{3}H-R2858 (5×10^{-11}–10^{-7}M) alone or in the presence of 100-fold unlabelled E_2 for 2 min–2 h at 0 °C and 37 °C. After incubation the MCF-7 cells were washed three times in MEM, then washed with an antibody generated against E_2 (Roussel ACE$_2$-3341 diluted 1/500 in MEM) and then spun down in 0.3 M sucrose (MEM). The use of antibody against E_2 greatly reduced background. The pellet of cells was then mounted on a stud with OCT (Tissue-Tek II), frozen in liquefied propane and processed for autoradiography.

After 2 h of incubation at 0 °C, approximately 76% of the grains were located over the nucleus (Fig. 4), whereas at 37 °C more than 90% were over nuclei. Nuclear localisation was found at 0 °C with as little as 2-min of incubation. The ratio of grains over the nucleus and cytoplasm over the concentration of ^{3}H-ligand used remained constant. However, at the lower concentrations of ligand the amount of exposure necessary to observe localisation increased that is, the quantity of observed silver grains was dose dependent). This confirms the biochemical studies of nuclear localisation of receptor at 0° C in MCF-7 cells[7].

Biochemical studies: problems with loosely trapped steroid

Autoradiograms from rat uteri exposed to ^{3}H-steroid at 0 °C for 5 min and 2 h, showed that a 5-min incubation at 2 °C would not result in the filling of 60–75% of the cytoplasmic receptors, as originally reported[11]. To resolve the discrepancy between the autoradiography in our study and the previous biochemical data, we investigated whether the binding observed previously[11] could have been the result of steroid–receptor interactions after the initial incubation period, that is during centrifugation at 225,000*g* for 1 h. In our first experiment uteri were incubated for 5 min at 0 °C with ^{3}H-E_2 (5×10^{-9}M) or ^{3}H-E_2 (5×10^{-9} M) + unlabelled E_2 (5×10^{-7} M). After 5 min the uteri were washed three times with buffer and then homogenised. In a second group, ^{3}H-E_2 (5 nM) was added to the uteri, and they were homogenised immediately (0.1 M PO_4, 0.01% monothioglycerol, 5 mM Mg and 10% glycerol, *p*H 7.4 at 0 °C). The homogenates were then spun at 105,000*g* for 1 h, and the resultant supernatants (cytosol) were withdrawn for either a hydroxyapatite assay or sucrose gradients (see below). Protein concentrations were determined by the method of Lowry[13].

For the hydroxyapatite assay[14], 200 μl of cytosol was mixed with 200 μl of hydroxyapatite suspension (1 volume dry powered DNA-grade hydroxyapatite (Bio-Rad) made up to 2 volumes with buffer). Tubes were mixed frequently for 30 min and then centrifuged for 2 min at 800*g*. The supernatant was discarded and the hydroxyapatite was washed three times with 2 ml of buffer with the same centrifugation each time. Then 1 ml of 100% ethanol was added to the hydroxyapatite and the mixture was incubated at room temperature for 1 h with frequent remixing to extract ^{3}H-steroid. Tubes were then centrifuged for 2 min at 200*g* and the supernatant was counted with 5 ml of ACS scintillation fluid (Amersham/Searle).

For the sucrose gradients, 200 μl of cytosol were layered on a density gradient of 5–30% sucrose in buffer. The gradients were centrifuged at 54,000 r.p.m. on a Beckman SW60 rotor for 16 h. Fractions of 200 μl were collected from the bottom and counted with 5 ml of ACS scintillation fluid.

In both the hydroxyapatite and the sucrose density gradient assay we obtained specific binding (8S) in cytosols from tissue which had been incubated with 5 mM ^{3}H-E_2 and in those not labelled until just before homogenisation (data not shown). Labelling with 5 nM just before homogenisation resulted in a fourfold increase over that seen in the tissue that was incubated and then washed.

In a second experiment we varied the concentration of the ^{3}H-E_2 added just before homogenisation and found that 1.25 nM added just before homogenisation was equivalent to a 5-min incubation with 5 nM. Similar results were obtained with the hydroxyapatite.

In a third experiment we prepared cytosol by centrifugation at 250,000*g* and did not add labelled steroid until just before centrifugation (that is, after homogenisation). Again we obtained as 8S peak indicating that the steroid-receptor interaction took place in the centrifuge. Therefore, ^{3}H-E_2 can bind to free receptor in the centrifuge tube. Thus, any loosely trapped steroid outside the target cells of standardly washed uteri could bind to free receptor during the tissue homogenisation and the

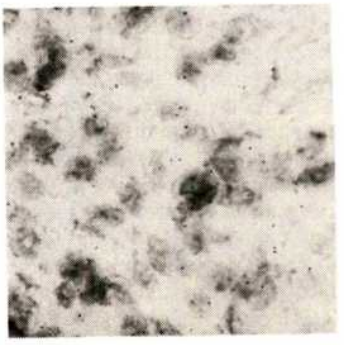


Fig. 2 Autoradiogram of rat uteri from the centre of a section incubated for 2 h at 0 °C with ^{3}H-oestradiol-17β. Note the paucity of silver grains. Stained with methylgreen-pyronin, 40-d exposure, 4-μm section, ×500.

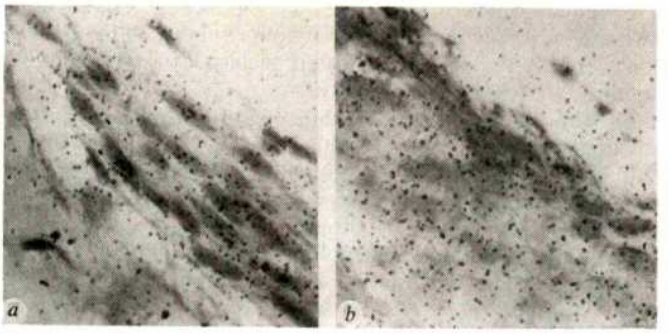


Fig. 3 Autoradiograms of rat uteri from the extreme periphery of the sections. The serosal surface is to the right in both autoradiograms. *a*, Incubated at 0 °C for 5 min with ^{3}H-R2858 (5×10^{-9}M); *b*, incubated at 0 °C with ^{3}H-R2858 (5×10^{-9}M) + unlabelled R2858 ($5\times10^{-}$7M). Stained with methylgreen-pyronin, 3.5-month exposure, 4-μm section, ×500.

preparation of cytosol in accord with the demonstration[15], with the use of cold chase experiments, that most of the binding reported earlier[11] occurred after the initial incubation period. The autoradiographic data presented here and the early biochemical data[15], together with our biochemical data therefore indicate that most of the binding of ^{3}H-E_2 to the receptor reported earlier[11] can be accounted for not in the incubation period, but during preparation of the cytosol. In addition, our autoradiographic data point to a nuclear localisation of ^{3}H-E_2 or ^{3}H-R2858 in the smooth muscle cells at the periphery of uteri incubated for 5 min at 0 °C, thus suggesting the presence of unbound nuclear receptor. The only evidence for the two-step cytoplasmic model for the intracellular translocation of steroids in the rat uterus is the observation that on homogenisation in a buffer most of the binding activity appears in the cytosol.

Equilibrium: mechanism of action of steroids

One mechanism which would explain the appearance of all binding activity in the cytosol after homogenisation of untreated uteri (when much of it was of nuclear origin *in vivo*) is a change in the free water content of the cytoplasmic compartment. That is, if the receptor were in equilibrium between the nucleus and cytoplasm, partitioned in some way according to the free water present in each compartment, homogenisation of uteri in buffer would artificially increase the water content of the non-nuclear compartment, that is, the cytosol, in agreement with the water equilibrium model[16]. To reestablish the original equilibrium much of the nuclear receptor would have to enter the cytosol. We tested this hypothesis with the following experiments.

Twenty-four uteri from 21–23-d-old Sprague–Dawley rats were homogenised at 0 °C in 3.5 ml of phosphate buffer (5 mM sodium phosphate, 0.01% mM monothioglycerol, 10% glycerol, *p*H 7.4) or Tris buffer (0.01 M Tris, 0.0015 M EDTA, 0.01% monothioglycerol, 10% glycerol, *p*H 7.4). Aliquots of the homogenate were distributed to six tubes and the following volumes of buffer were added to the tubes: 0, 0.5, 1.5, 4.5, 13.5 and 40.5 ml. The tubes were allowed to stand at 0 °C for 5 min and then centrifuged at 800*g* for 10 min. The resultant supernatants were decanted off. The first three tubes containing the supernatant were brought to a volume of 3 ml with buffer to insure an adequate volume of cytosol for the assay and all supernatants were then spun at 105,000*g* for 1 h. The resultant supernatants (cytosols) were then assayed for oestrogen receptors (see below). The nuclei from the 800*g* spin were extracted with either 3 ml of buffer or 3 ml of 0.6 M KCl in buffer for 60 min at 0 °C and then centrifuged at 800*g*. The supernatants were decanted and assayed for oestrogen receptor. For reasons that will become obvious it was considered imperative that the nuclear preparation remain as crude homogenates.

Samples of 200 μl of cytosol or KCl extract or buffer extract of nuclear pellet were incubated with 50 μl of [1,2,6,7-^{3}H]E_2 (109 Ci mol^{-1}, final concentration 5×10^{-9}) alone or with ^{3}H-E_2 + 5×10^{-7} unlabelled E_2 for 3 h at 0 °C. After incubation, the tubes were assayed by the hydroxyapatite method[14].

Figure 5 shows the effect of adding increasing volumes of buffer before separation of cytosol from the nuclear fraction (note that all values were converted to total amount of receptor in either nucleus or cytoplasm). Increasing the amount of buffer decreases the amount of receptor in the nucleus. We have repeated this experiment seven times with similar results. The shape of the curve is highly suggestive of a simple equilibrium or of a desorption of receptor from the nuclear pellet (the autoradiographic data suggest that some receptor is of nuclear origin).

We assume the following model. Nuclear receptors (R_n) are in equilibrium with cytoplasmic receptors (R_c) *in vivo*. The equilibrium may be represented by a distribution coefficient identifying the components of the partitioning of the receptors between the nucleus and the cytoplasm.

$$R_c \leftrightarrow R_n \tag{1}$$

and

$$D = \frac{R_n/V_n}{R_c/V_c} \tag{2}$$

where V_n and V_c are the nuclear and cytosol fluid volumes, respectively. Introducing $R_T = R_n + R_c$ and solving this equation for R_n yields:

$$R_n = \frac{R_T}{VcK + 1} \tag{3}$$

where

$$K = \frac{1}{DVn} \tag{4}$$

The limits of equation (3) indicate the direction of flow of the receptors between nucleus and cytoplasm. As $V_c \to 0$, $R_n \to R_T$

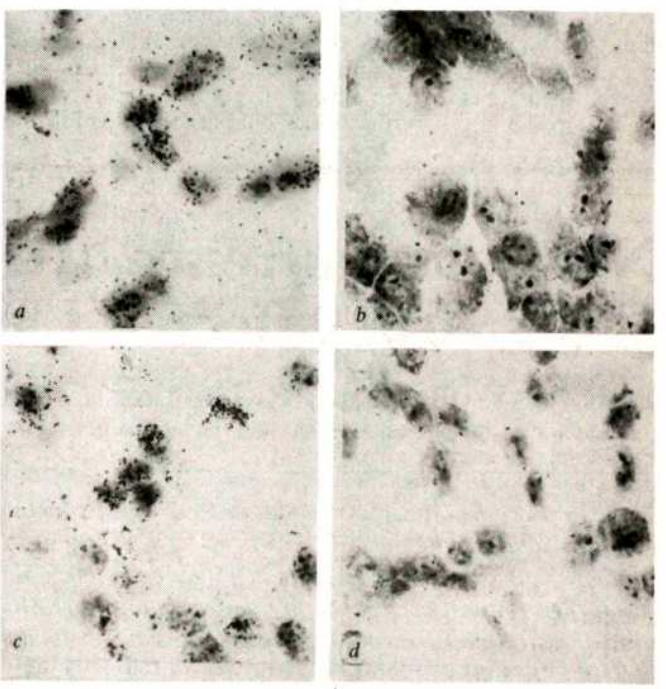


Fig. 4 Autoradiograms of MCF-7 cells. *a*, Cells incubated for 2 h with ^{3}H-oestradiol (1×10^{-9}M) at 0 °C, and *c*, at 37 °C. *b*, Cells incubated for 2 h with ^{3}H-oestradiol (1×10^{-9}M) − unlabelled oestradiol (1×10^{-7}M) at 0 °C, and *d* at 37 °C. The predominant localisation is nuclear at both 0 °C and 37 °C. The large black dots seen in the nuclei of unlabelled cells (*b* and *d*) are nucleoli. Stained with methylgreen-pyronin, 2-week exposure, 4-μm sections, ×900.

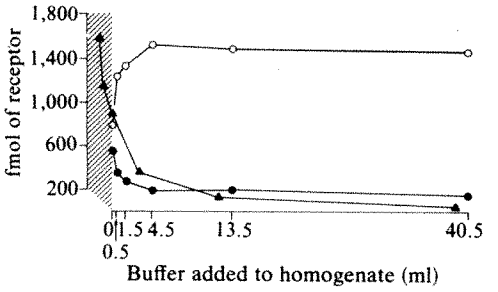


Fig. 5 Amount of apparent nuclear receptor (●), and amount of apparent cytoplasmic receptor (○) plotted as a function of volume of buffer added to homogenate. At 0 added volume the slope of the curve is quite steep. The theoretical values for nuclear receptor that would be obtained if $R_T - 1{,}588$ fmol and $K = 0.75$ in equation (3) are plotted as a function of the cytosolic volume (V_c) (▲). Because we can never approach $V_c = 0$ or the *in situ* volume of the cytoplasm experimentally, we have moved the x-axis to the right an arbitrary amount and put this part of the theoretical curve under cross-hatching.

(*in vivo*) and as $V_c \to \infty$, $R_n \to 0$ (in typical *in vitro* experiments in which large volumes of buffer are used for homogenisation). R_n is measured as c.p.m. in the nuclear pellet and V_c is the total added volume of the homogenate. In Fig. 5 we plot R_n and R_c as a function of V_c. The third plot in Fig. 5 contains points calculated from equation (3) with $K = 0.75$.

For the immature rat uterus, the distribution constant is high. We expect that this constant will vary with different steroids and different tissues. This model provides for the first time a mechanism for the translocation of cytoplasmic (*in vivo*) or cytosolic (*in vitro*) receptor to the nucleus (no mechanism has been found which is compatible with the two-step cytoplasmic model[17]).

In this scheme, free steroids enter both the cytoplasm and the nucleus of a target cell and bind to unoccupied receptors. *In vivo* or *in vitro* at 37 °C, the receptors are activated *in situ* and the nuclear fraction of steroid–receptor complexes binds to chromatin and thus precipitates out of solution (that is, one has to extract with KCl to get them back in solution). The nuclear volume then appears to be devoid of receptors and receptors move into the nucleus from the cytoplasm to reestablish the initial equilibrium (translocation). This model agrees very well with the published data, and in fact the equilibrium model was considered in the past but rejected because of the inability to demonstrate free nuclear receptor[18].

As with many of the biochemical studies dealing with the location of unbound steroid receptors[15], our data are subject to different interpretations. Alternative explanations for the results of our dilution experiments are (1) that the initial homogenisation of the tissue in hypotonic buffer caused swelling of the nuclei and an initial shift of cytoplasmic receptor into the nuclear compartment and that subsequent dilutions just redistributed the receptor back into the cytoplasmic compartment, or (2) that the increased nuclear receptor with a concentrated homogenate is due to nonspecific adsorption of cytoplasmic receptor to the nuclei. While we feel that both these explanations are possible, we consider them improbable. We feel that of all previously used techniques, autoradiography is the least susceptible to redistribution artefacts. The autoradiographic data presented here for the uterus suggest that there are bound nuclear receptors at 0–4 °C which appear in the cytoplasmic compartment on homogenisation[15].

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Natural occurrence of 2-5A in interferon-treated EMC virus-infected L cells

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Until now the interferon-mediated 2′–5′ adenine oligonucleotide inhibitors (2–5A) of cell-free protein synthesis have not been detected in intact cells. Here we report their natural occurrence in interferon-treated, EMC virus-infected mouse L cells in amounts consistent with the idea that they play a part in the inhibition of virus growth.

SOME years ago we demonstrated an interferon-mediated inhibition of cell-free protein synthesis in extracts prepared from interferon-treated, virus-infected cells[1,2]. Subsequently we reported that protein synthesis in cell-free systems from interferon-treated cells shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA)[3]. On the basis of these observations and the known effects of dsRNA in the intact cell and cell-free system[4–6], we suggested[3] that the production of viral dsRNA in response to infection (even with a DNA virus[7]) could bring about a general inhibition of protein synthesis in interferon-treated cells such as is indeed observed, for example, in interferon-treated, vaccinia virus-infected mouse L cells[8,9]. The cell dies but little virus is produced: an effective way of limiting a natural infection. Alternatively, the triggering by dsRNA of a more transient or selective inhibition could result in the degradation of viral nucleic acid but allow the ultimate recovery of the cell. The enhanced sensitivity of cell-free protein

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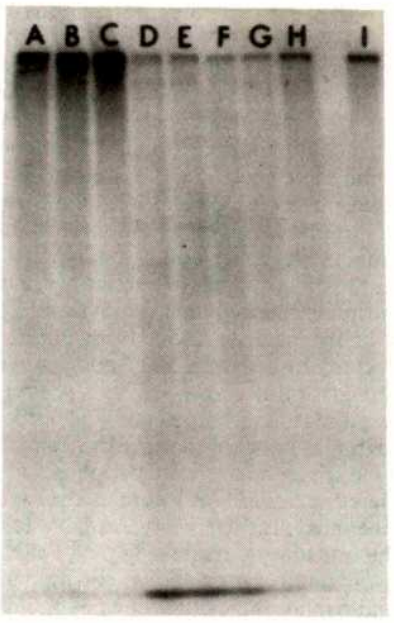


Fig. 1 Recovery of 2-5A from intact cells. 2-5A was assayed by its ability to activate a nuclease and cause the degradation of added ^{32}P-RNA in rabbit reticulocyte lysates[23]. Autoradiographs of SDS polyacrylamide gel analyses of the ^{32}P-RNA are shown. Comparison of 2-5A activity isolated from control (A), EMC infected (B), interferon-treated (C) and interferon-treated, EMC virus-infected (D–H) cells at overall dilutions of 6 (A–D) and 18, 54, 162 and 486 (E–H) fold, respectively. I, control (no inhibitor). In typical assays of this type using standard preparations of 2-5A, nuclease activity is clearly detectable down to a final concentration of $\leq 2.4 \times 10^{-9}$ M (Fig. 3*a*, tracks 19–23). Mouse L cells (300 ml, 0.75×10^6 cells per ml) were treated for 17 h at 37°C with 400 reference units (20 effective units) per ml of partially purified (5×10^7 reference units per mg of protein) mouse interferon, infected with 20 PFU per cell of EMC virus and incubated for 4 h at 37 °C. Control cells with or without interferon treatment or EMC infection were incubated in parallel. Cells were collected and washed, packed cells were lysed into an equal volume of 0.5% NP40 in 10 mM HEPES buffer, *p*H 7.5, 90 mM KCl, 1.5 mM magnesium acetate. The 100,000*g* supernatant fractions (500 μl) were heated to 95 °C for 5 min, centrifuged to remove denatured protein and the supernatants fractionated on DEAE Sephadex[28]. Fractions corresponding to 2-5A were eluted with 1 M NH_4HCO_3, lyophilised extensively to remove bicarbonate and redissolved in 20 μl of H_2O before assay. In the nuclease assays here and in Figs 2 and 3, 30–300 ng (equivalent to 3,000–5,000 c.p.m. at a specific activity of 10^4–10^5 c.p.m. per μg) of ^{32}P-labelled polyadenylated mRNA (ref. 20) or 28S rRNA from Ehrlich ascites tumour cells were added per 12.5-μl assay. (Identical results were obtained throughout with the two types of RNA.)

synthesis to dsRNA showed the same interferon dose–response curve as did the antiviral effect in the intact cell, indicating that the two effects might indeed be closely related[3]. The basis for the effect of dsRNA was, therefore, investigated.

In the cell-free system two distinct mechanisms seem to be involved: (1) phosphorylation of the protein synthesis initiation factor eIF2 by an interferon and dsRNA mediated kinase; and (2) degradation of mRNA by a nuclease(s) activated by sub-nanomolar levels of an unusual oligonucleotide pppA2′p5′A2′p5′A (and related oligomers collectively referred to as 2-5A) synthesised by an interferon and dsRNA-mediated synthetase[10–18]. In intact cells, 2-5A, when artificially introduced, has also been shown to be effective in the activation of a nuclease(s) and consequent inhibition of protein synthesis and virus growth[19–21]. Both in the cell-free system and when introduced into intact cells 2-5A is unstable and in the absence of a 2-5A regenerating system the activation of the nuclease and inhibition of protein synthesis are transient[18,20–22]. All of these results are consistent with a possible role for 2-5A in the inhibition of virus replication in the interferon-treated cell, but they do not provide direct evidence for it. More particularly, there has been no direct evidence for the natural occurrence of 2-5A, which has only been detected after synthesis in crude cell extracts or with the partially-purified synthetase. It has remained possible, therefore, that it is an artefact of cell-free systems. We recently reported that 2-5A deliberately introduced into cells can be recovered from them[20]. Using the same methods for the recovery of 2-5A here we report its natural occurrence in interferon-treated, encephalomyocarditis (EMC) virus-infected cells in amounts consistent with the idea that it plays a part in the inhibition of virus growth in these cells.

Presence of 2-5A in extracts from interferon-treated, EMC virus-infected L cells

Extracts were prepared from interferon-treated and control cells 4 h postinfection with EMC virus and centrifuged at 2,000 or 100,000*g*. The supernatant fractions were either precipitated with trichloroacetic acid (TCA, for the 2,000*g* supernatant) or heated to 95 °C for 5 min (for the 100,000*g*), centrifuged to remove denatured protein and purified on DEAE Sephadex to remove the majority of contaminating mono- and oligonucleotides.

The fractions corresponding to the position at which 2-5A would have been expected to elute were pooled, lyophilised, redissolved in a small volume and assayed for 2-5A. Two types of assay were used: activation of a nuclease in the rabbit reticulocyte lysate and inhibition of protein synthesis in EMC RNA-programmed cell-free systems from Ehrlich ascites tumour (EAT) cells[23]. In the first of these (Fig. 1A) ^{32}P-labelled mRNA was incubated in a rabbit reticulocyte lysate in the presence or absence of added test material. Whereas the addition of material from control cells plus or minus EMC infection or from interferon-treated cells was without significant effect (Fig. 1, A–C respectively), that from interferon-treated, EMC virus-infected cells showed evidence for activation of a nuclease(s) over a range of dilutions from 6 to 486-fold (Fig. 1, D–H). In accord with this, of these extracts, only that from interferon-treated, EMC virus-infected cells also inhibited protein synthesis at similar dilutions in the cell-free system. In an additional control experiment an extract from such cells, but not from a mixture of equal volumes of interferon-treated cells and EMC virus-infected cells, was similarly effective in activating a nuclease in the reticulocyte system. In this latter experiment the interferon-treated, EMC vius-infected cells and the mixture (interferon-treated cells plus EMC-infected cells) were each lysed with NP40 on ice and immediately precipitated with TCA. This was done to exclude the formal possibility that any 2-5A detected could have been synthesised during the processing of the extracts. (In fact, the extract prepared from the interferon-treated, EMC virus-infected cells in the latter more rapid fashion contained if anything slightly greater activity than did

Table 1 Trimer and tetramer triphosphate components of 2-5A recovered from HPLC of an extract from interferon-treated, EMC virus-infected L cells assayed by inhibition of cell-free protein synthesis

Dilution into the cell-free system	^{14}C-amino acid incorporation in the presence of dialysed and concentrated HPLC fractions (c.p.m. per 10 μl)*	
	25–29 (putative trimer, triphosphate)	35–39 (putative tetramer, triphosphate)
62.5	3,700	4,900
125	6,250	5,714
375	7,830	7,800
1,250	14,250	14,316
3,750	16,480	16,500

Pooled fractions 25–29 (750 μl) and 35–39 (750 μl) from the HPLC analysis of the interferon-treated, EMC virus-infected cell material shown in Fig. 2*b* were each dialysed against four changes of 100 volumes of H_2O at 4 °C, lyophilised, redissolved in 20 μl of H_2O and assayed in an EMC RNA-programmed EAT cell-free system as described in Fig. 4. Standard trimer and tetramer preparations (750 μl of 1 μM in 0.2 M ammonium phosphate, *p*H 7.0) processed in parallel were each recovered in 80% yield. Non-phosphorylated A2′p5′A2′p5′A (1 μM) was added as 'carrier' before dialysis: parallel controls confirmed that it was without effect in the cell-free system.

* Incorporation in the absence of added inhibitor was 17,100.

those prepared by the longer method involving the initial isolation of a 100,000*g* supernatant.) The material in the interferon-treated, infected cell extract responsible for the activation of the nuclease in the reticulocyte lysate was sensitive to digestion with bacterial alkaline phosphatase and snake venom phosphodiesterase, but resistant to digestion with T2 and micrococcal nucleases, as would be expected for 2-5A[24]. Further evidence for the identity of this material as 2-5A came from its performance on HPLC in comparison with appropriate standards.

Characterisation of 2-5A by HPLC and comparison of the relative activities of the trimer and tetramer in the rabbit reticulocyte and EAT cell-free systems

2-5A synthesised with the synthetase from interferon-treated mouse L cells or rabbit reticulocytes consists of a mixture of oligonucleotides of the general formula ppp(A2′p)$_n$A with the trimer as the predominant species[12,25]. The individual components, trimer, tetramer and pentamer ($n = 2$–4, respectively) were isolated by chromatography on DEAE cellulose[25] and subjected to HPLC. In Fig. 2*a* the pattern obtained with a recombined mixture of these standard preparations is presented. Peaks 7 to 9 correspond to the trimer, tetramer and pentamer triphosphates, respectively. All preparations also contain smaller and variable amounts of the corresponding di- and monophosphates (peaks 4–6 and 1–3, respectively)[25]. These are thought to represent breakdown products of the predominant triphosphates. (The terms trimer, tetramer and pentamer will be used to denote preparations of this type consisting largely of the triphosphates, but also containing smaller amounts of the di- and monophosphates. The more complete nomenclature, for example trimer triphosphate, will be used to identify the individual species.) It has previously been shown that the trimer diphosphate (but not the monophosphate) has essentially the same specific biological activity as the corresponding triphosphate (ref. 25 and L. A. Ball, personal communication). As will be seen below, the same seems to be true for the corresponding tetramer and pentamer species. It is not known whether the diphosphates are active *per se* or are simply efficiently rephosphorylated in the assay systems used.

An analysis of the putative 2-5A from interferon-treated, EMC virus-infected cells (to which ATP and p$_4$A were added as optical density markers) is shown in Fig. 2*b*. Predictably, too little 2-5A was present for it to be clearly identified by optical density (although it is possible that peaks X and Y do indeed correspond to the trimer and tetramer triphosphates). Accordingly, appropriate fractions (1–53) were analysed for biological activity using the most sensitive assay available to us; nuclease activation in the reticulocyte lysate (Fig. 2*c*). Peaks of nuclease activity were obtained in positions corresponding to the tetramer di- and triphosphates (fractions 6–8 and 35–41) and the pentamer di- and triphosphates (fractions 11–13 and 45–47).

The apparent absence of trimer in the above preparation was curious but the explanation for it became clear from an analysis of the activity of appropriate standards. It seems that the trimer tri- and diphosphates (whether chemically or enzymically synthesised) are relatively poor activators of the nuclease and inhibitors of protein synthesis in rabbit reticulocyte lysates,

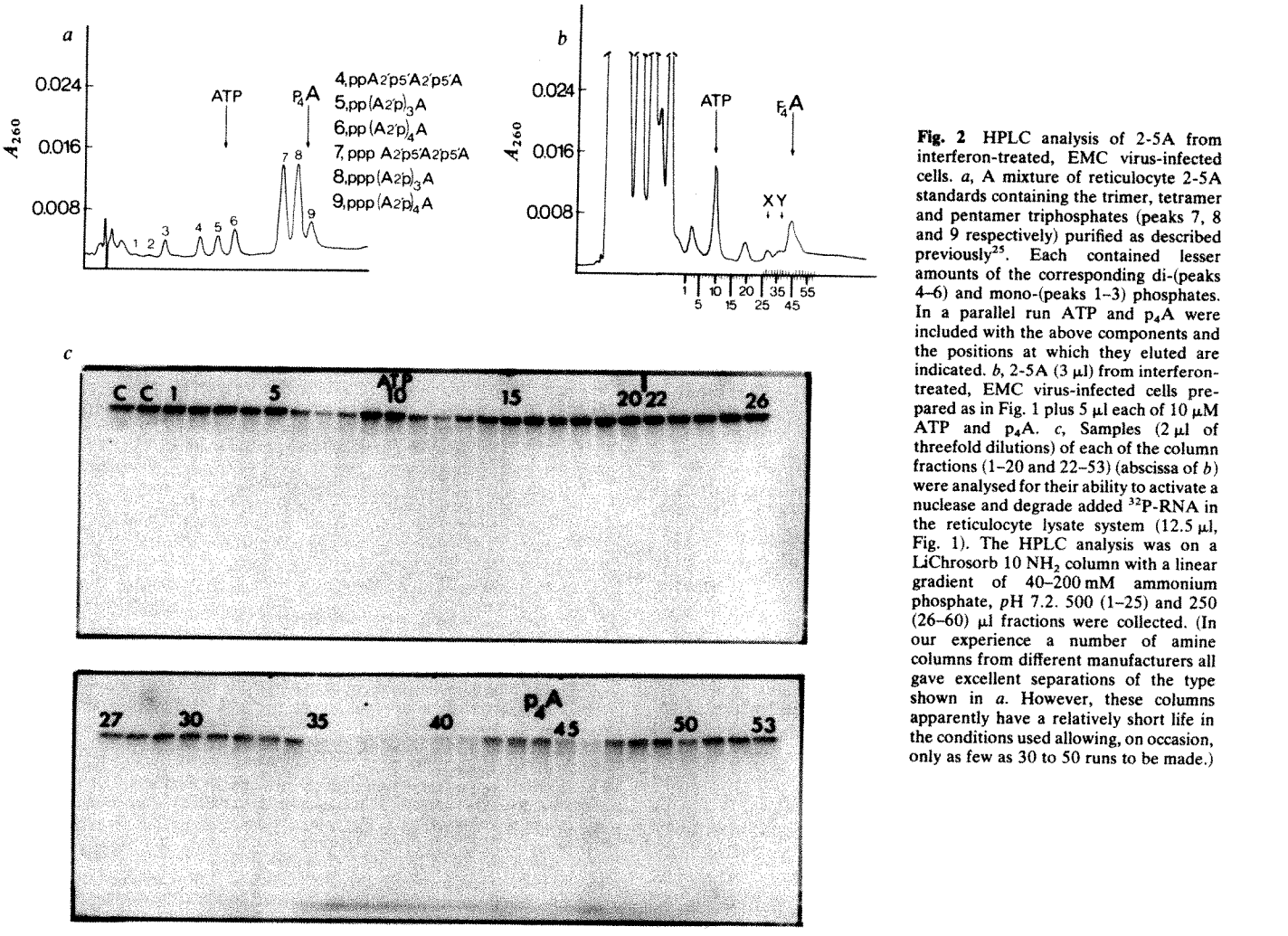


Fig. 2 HPLC analysis of 2-5A from interferon-treated, EMC virus-infected cells. *a*, A mixture of reticulocyte 2-5A standards containing the trimer, tetramer and pentamer triphosphates (peaks 7, 8 and 9 respectively) purified as described previously[25]. Each contained lesser amounts of the corresponding di-(peaks 4–6) and mono-(peaks 1–3) phosphates. In a parallel run ATP and p$_4$A were included with the above components and the positions at which they eluted are indicated. *b*, 2-5A (3 μl) from interferon-treated, EMC virus-infected cells prepared as in Fig. 1 plus 5 μl each of 10 μM ATP and p$_4$A. *c*, Samples (2 μl of threefold dilutions) of each of the column fractions (1–20 and 22–53) (abscissa of *b*) were analysed for their ability to activate a nuclease and degrade added ^{32}P-RNA in the reticulocyte lysate system (12.5 μl, Fig. 1). The HPLC analysis was on a LiChrosorb 10 NH$_2$ column with a linear gradient of 40–200 mM ammonium phosphate, *p*H 7.2. 500 (1–25) and 250 (26–60) μl fractions were collected. (In our experience a number of amine columns from different manufacturers all gave excellent separations of the type shown in *a*. However, these columns apparently have a relatively short life in the conditions used allowing, on occasion, only as few as 30 to 50 runs to be made.)

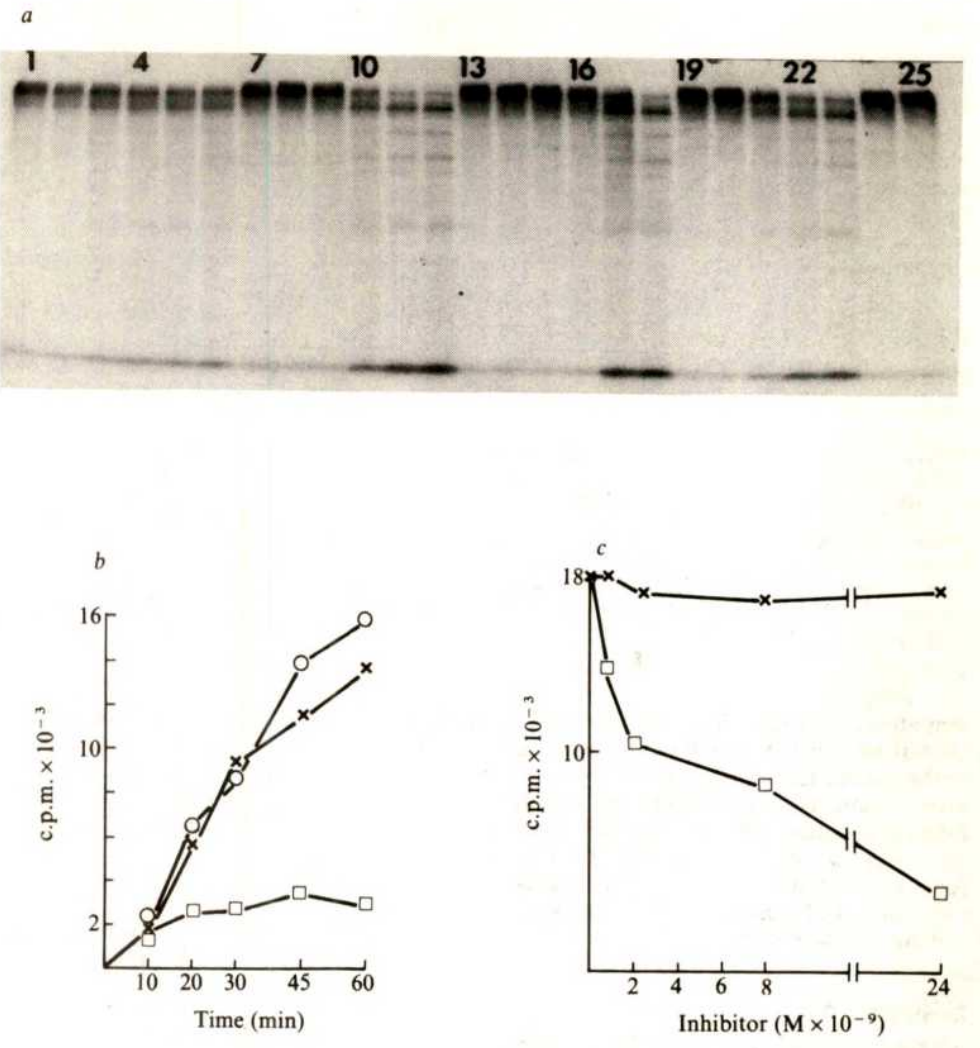


Fig. 3 Comparison of the activities of the trimer, tetramer and pentamer components of reticulocyte 2-5A in the reticulocyte lysate system. *a*, Activation of a nuclease. Tracks 1–6: 2.4, 8, 24, 80, 240 and 800×10^{-9} M trimer. Tracks 7–12 and 13–18: O.024, 0.08, 0.24, 0.8, 2.4 and 24×10^{-9} M tetramer and pentamer respectively. Tracks 19–23: 0.24, 0.8, 2.4, 8 and 24×10^{-9} M 2-5A (unfractionated). Tracks 24 and 25: no inhibitor controls. Assays were carried out as described in Fig. 1. *b*, *c*, Inhibition of ^{35}S-methionine incorporation in micrococcal nuclease treated rabbit reticulocyte lysate systems[29] programmed with EMC RNA[23]. *b*, Time course of incorporation in the absence (○) and presence of 8×10^{-9} M trimer (×) and tetramer (□). *c*, Inhibitory activity of different concentrations (abscissa) of trimer (×) and tetramer (□). The ordinates give the ^{35}S-methionine incorporated (c.p.m. per 10-μl sample) into acid insoluble polypeptide with time of incubation at 30 °C (abscissa) in (*b*) and at 60 min in (*c*). The reticulocyte, trimer, tetramer and pentamer preparations were those analysed in Fig. 2*a*. The concentrations of these and of the unfractionated preparation of 2–5 A are given in AMP equivalents. (1 ml of 1 mM inhibitor was taken to have an absorbance of 15 at 260 nm measured in a 1 cm pathlength cell.)

although apparently fully active in this respect in other systems (refs 12 and 25 and Fig. 4 below). The relative activities of the trimer, tetramer and pentamer in the activation of a nuclease in the reticulocyte lysate are compared in Fig. 3*a*. The tetramer and pentamer are both capable of clear-cut activation of a nuclease at final concentrations of 10^{-9} M. (The concentrations of the different species of 2-5A are given in AMP equivalents throughout.) Indeed, with fresh preparations of high specific activity RNA requiring the addition of smaller amounts of RNA, activation of nuclease can be detected with as little as 0.024×10^{-9} M of these components in this type of assay. Significantly, higher concentrations of the trimer are required ($>8 \times 10^{-9}$ M) and, more importantly, even with these higher concentrations the activation of the nuclease seems to be very much less efficient (Fig 3*a*, tracks 1–6). In accord with this, 0.8 and 8×10^{-9} M tetramer (□) were sufficient to give detectable and substantial inhibitions respectively of amino acid incorporation in an EMC RNA-programmed reticulocyte lysate, whereas similar or greater concentrations of trimer (×) were without effect (Fig. 3*b*, *c*). In contrast the trimer (×) and tetramer (□) appeared almost equally effective in activating nuclease(s) (Fig. 4*a*, *b*) or inhibiting EMC RNA-programmed protein synthesis (Fig. 4*c*) in the EAT cell-free system.

The basis for the ineffectiveness of the trimer in the reticulocyte cell-free system is not known. It does not seem to be the result of a more rapid degradation of this component in this system and most probably, therefore, reflects a preference of the activatable nuclease for the tetramer or higher oligomers. Whatever the explanation, it is clear that the failure to detect trimer in the HPLC analysis of the 2-5A present in the interferon-treated, EMC virus-infected cell extracts described in Fig. 2*b* could simply have reflected the choice of the reticulocyte as the assay system. This was shown to be the case by assaying appropriate fractions from the HPLC analysis (Fig. 2*b*) for the ability to activate a nuclease and inhibit protein synthesis in the EAT system. In a nuclease assay measuring conversion of ^{3}H-labelled VSV mRNA to acid solubles of the type shown in Fig. 4*a*, *b*, peaks were obtained in fractions 1 (corresponding to the trimer diphosphate), 7 (tetramer diphosphate), 26–28 (trimer triphosphate) and 36–38 (tetramer triphosphate), the results being consistent with there being, if anything, slightly more trimer than tetramer present (data not presented). The slightly lower sensitivity of the ^{14}C-labelled amino acid incorporation assay combined more importantly with its higher sensitivity to the 'non-specific' inhibitory effects of the salt gradient used in the elution from HPLC, made it impossible to detect convincingly even the triphosphorylated trimer and tetramer peaks by direct analysis of the column fractions in this type of assay. They were readily detected, however, in apparently identical amounts when appropriate column fractions were pooled, dialysed, concentrated and analysed in such systems (Table 1).

It can be concluded, therefore, that the triphosphorylated trimer, tetramer and pentamer components at least of 2-5A are present in interferon-treated mouse L cells 4 h postinfection with 20 plaque forming units (PFU) per cell of EMC virus. It is possible that smaller amounts of the diphosphates are also present in the intact cell, but it seems probable that these reflect partial breakdown products of the corresponding triphosphates. If higher oligomers are present they are not detectable with the

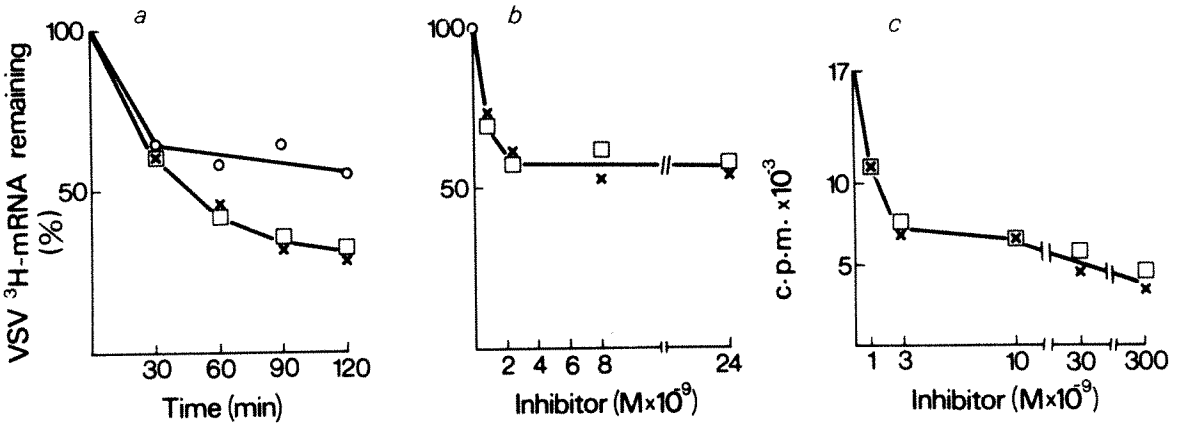


Fig. 4 Comparison of the activities of the trimer and tetramer components of reticulocyte 2-5A in EAT cell-free systems. *a*, *b*, Activation of a nuclease. Breakdown of VSV ^{3}H-mRNA on incubation in an EAT cell-free system: *a*, alone (○) or with 8×10^{-9} M trimer (×) or tetramer (□) or, *b*, with different concentrations (abscissa) of trimer (×) or tetramer (□). VSV ^{3}H-mRNA (4×10^5 c.p.m. per μg) was added to a final concentration of 2 μg ml^{-1} and incubated in identical conditions to those previously described for L cell-free systems[18] at 30 °C for the times indicated in (*a*) and 120 min in (*b*). The ordinates give the residual acid insoluble radioactivity as a percentage of the zero time value (2,600 c.p.m. per 4-μl sample) in (*a*) and of the 120-min control (no inhibitor) value (3,500 c.p.m. per 10-μl sample) in (*b*). (The 100% point in (*b*) corresponds to the 120 min control (○) point in (*a*).) *c*, Inhibition of amino acid incorporation by different concentrations (abscissa) of the trimer (×) and tetramer (□) in an EMC RNA-programmed EAT cell-free system prepared and incubated for 120 min at 30 °C as described for similar systems from mouse L cells[18,30]. The ordinate gives the incorporation of a mixture of ^{14}C-amino acids into acid insoluble polypeptide (c.p.m. per 10-μl sample). The trimer and tetramer preparations were the same as those used in Figs 2*a* and 3. Their concentrations are given in AMP equivalents as described in Fig. 1.

procedures used here. The heterogeneity in the length of the 2-5A synthesised in cell extracts has been one of the odd aspects of the system for which as yet there is no explanation. This, perhaps more than anything else, emphasised the possibility that 2-5A, in the form isolated, might be no more than an intriguing artifact of cell-free systems. The discovery of similar heterogeneity here in the intact cell argues against this and, although providing no explanation for the phenomenon, suggests that it may have some significance.

Discussion

We have previously shown that 2-5A artificially introduced into cells is capable of activating a nuclease(s) and inhibiting protein synthesis and virus growth[19–21]. Here we have demonstrated the natural occurrence of 2-5A in amounts sufficient for it to be responsible for a similar sequence of events in interferon-treated, EMC virus-infected L cells. Assuming 50% recovery of the 2-5A throughout the isolation procedure (which seems reasonable from appropriate control experiments), the yields obtained in the four experiments completed have been consistent with concentrations of $20–200 \times 10^{-9}$ M 2-5A in the interferon-treated, EMC virus-infected cells from which the extracts were made. Preliminary evidence has also been obtained for an enhanced rate of breakdown of preformed RNA in these cells consistent with the activation of a nuclease (unpublished data). The relative importance of this mechanism, however, compared with any effect of the interferon and dsRNA mediated kinase, or alternative mechanism(s) not mediated through dsRNA, in this and other interferon-treated, virus-infected cell systems remains to be established.

Here we have detected 2-5A only in the extracts from interferon-treated, virus-infected cells, whereas the wide distribution and variation in amount of the synthetase has raised the possibility that it may have a wider significance in the regulation of cell growth or development[26]. The possible wider significance of the synthetase has been further emphasised by the recent demonstration by Ball that an enzyme which is almost certainly the 2-5A synthetase is capable (in cell extracts) of adding AMP in 2′-5′ linkage to a variety of important metabolites such as NAD, ADP ribose and AppppA (ref. 27). On the other hand, were 2-5A to have been present in physiological concentrations of less than 10×10^{-9} M in any of the above cells we would not have detected it with the procedures used. Clearly, a more sensitive and convenient assay for 2-5A and a much more detailed analysis of this and alternative systems will be required before the full significance of either 2-5A or its synthetase can be assessed. Meanwhile, however, it is unlikely from the results presented here that 2-5A is a mere artefact of the cell-free system and it is reasonable to assume that it plays some part in the inhibition of virus growth in the interferon-treated, EMC virus-infected cell.

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letters

Observations of a flaring X-ray pulsar in Dorado

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The γ-ray burst detector Konus[1], on the Venera 11 and Venera 12 spacecraft, detected on 5 and 6 March, 1979 two bursts of hard X rays originating from the same source. These events are quite unusual and of considerable interest. The burst of 5 March was very intense, particularly in the initial phase. This event was also observed by several other spacecraft[2]. The second burst on 6 March was considerably weaker. The observations reported here permitted us to obtain a detailed time structure of the bursts, to measure their energy spectra and to locate the source on the celestial sphere.

Figure 1 displays the time structure of the initial phase of the 5 March burst recorded on Venera 11 in the energy range 50–150 keV with a time resolution of 1/64 s. Note that all results obtained on Venera 11 and Venera 12 coincide completely. The burst has a very sharp onset. The initial, narrow structureless pulse is characterised by a very short rise time, ~15 ms, and a longer decay, ~150 ms. The peak count rate in the 50 cm^2 sensor attains 4×10^5 c.p.s. This means that during a short time (~0.1 s), the flux of hard X rays exceeded the diffuse cosmic background by a factor of 10^4. The time structure of the burst measured with resolutions of 1/4 and 1 s is shown in Fig. 2*a*, *b*. An analysis of these data clearly indicates that the radiation observed is that of an X-ray pulsar with a period 8.1 ± 0.1 s (ref. 3). The pulsed radiation is seen to exhibit a train of stronger pulses and another train of interpulses with a relative phase of 0.5. The level of intensity in the deep minima between the pulses apparently reflects a slowly varying component of radiation.

The most remarkable fact here is that the onset of radiation is almost instantaneous with a subsequent fast decay. The standard length of measurements (66 s) was found to be insufficient for this event; however, measurements carried out 6 min after the beginning of the burst revealed that by this time the intensity of radiation had decreased to background level.

Eight consecutive measurements of the energy spectra were carried out, each of 4 s duration. Only the first spectrum measured in the initial burst phase differs in shape from the others. Therefore, Fig. 3 presents the energy spectrum of the burst for the first time interval, 0–4 s (histogram *a*), and a spectrum averaged over the interval 4–32 s (histogram *b*). The energy spectra of this event differ from those of most of the γ-bursts[4]. The shape of the pulsed radiation spectrum is more similar to those of the spectra of continuously emitting X-ray sources, such as Cyg X−1, than to the substantially harder spectra of typical γ bursts. It may be approximated by the relationship $E^{-1}\exp(-E/kT)$, with $kT=30$ keV.

The spectrum of the initial burst phase has a harder tail, which is possibly associated with radiation of only the initial pulse. The spectral feature in the region 400–500 keV is a remarkable characteristic of this spectrum. The total identity of the spectra obtained on Venera 11 and Venera 12 completely eliminates the possibility that this feature is a result of random fluctuations. A more detailed analysis of the spectrum shows that the observed feature may be associated with the presence of a broad line at 430 keV with a FWHM of 30–40%. It is difficult to suggest an unambiguous explanation of the nature of this radiation component. Note, however, that 430 keV corresponds to the energy of the 511-keV annihilation line which has undergone a redshift of $(1-2GM/c^2R)^{1/2}$ in the gravitational field of a compact object with $M=1M_\odot$ and $R=10^6$ cm.

The average pulsed radiation flux with $E_\gamma>30$ keV is 1×10^{-5} erg cm^{-2} s^{-1}. At the peak of the initial pulse the flux is 1.5×10^{-3} erg cm^{-2} s^{-1}. Evaluation of the flux in the line with $E_\gamma\approx430$ keV yields 3×10^{-6} erg cm^{-2} s^{-1}, or, assuming the line radiation to be emitted only in the initial pulse, 5×10^{-5} erg cm^{-2} s^{-1}.

The location of this flaring X-ray pulsar, designated earlier FXP 0520−66 (ref. 3), is shown in Fig. 4. The readings of the Konus sensor unit with anisotropic angular sensitivity yield a fairly large error box with sides of about 3°. Intersection of this region with a narrow annulus obtained from the time-of-arrival difference between Venera 11 and Venera 12 gives the final box as a narrow annular band 4′ wide. Based on the latest spacecraft trajectory data, the position of this annulus on the celestial sphere is defined by the angular radius $68.528\pm0.030°$ with the centre at $\alpha=44.427°$, $\delta=-2.747°$ (1950.0). This position is in good agreement with other data. The arc of radius 68.528° passes 1.7 arc min to the south-east of the centre of the error box for this source given in ref. 2 − α = 5 h 25.92 min, $\delta=-66.122°$.

As mentioned above, the observed source was found to be recurrent. On the next day, 6 March, the Konus detector on both spacecraft recorded, after the same time interval $\Delta t=$ 14 h 25 min 46.15 s a short burst ~1.5 s long with the same soft

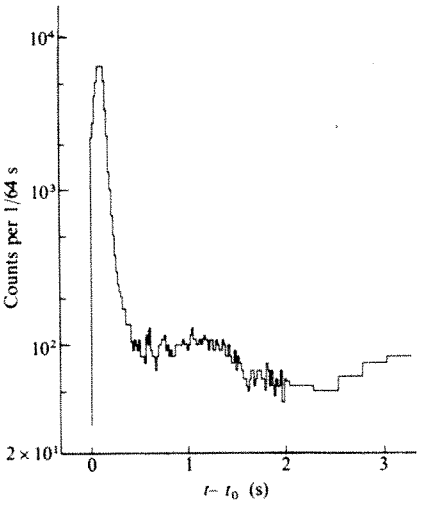


Fig. 1 Initial stage of the 5 March event as recorded on Venera 11. The data of Venera 12 are identical. Energy range covered 50–150 keV. Time resolution, 1/64 s. Burst onset time t_0. Venera 11: 15 h 51 min 39 s, 145 UT. Venera 12: 15 h 51 min 44 s, 350 UT.

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spectrum (Fig. 3, histogram *c*). Its intensity, however, was 100 times lower. Its time structure is shown in Fig. 2*c*, *d*, with results of source location determination presented in Fig. 4. For the 6 March event, the process responsible for the emission in the source was evidently weaker and longer than in the case of the 5 March burst. We can assume that the decaying pulsed radiation could have been accordingly weaker and, hence, not observable against the background present.

The most plausible model for the FXP0520−66 source seems to be a binary system containing a neutron star. The separation between the components should be sufficiently large to reduce the accretion rate to the level where there would be no observable radiation in the steady state. The neutron star should possess a strong magnetic field; indeed, the presence of two pulse trains in the burst implies that during accretion the plasma moves along the field lines with the emitting regions located near the magnetic poles. The origin of the non-stationary accretion resulting in a burst remains unclear. The characteristic rise time to almost full peak luminosity is small, less than 15 ms, implying that processes associated with the neutron star proper, such as the development of instabilities in the magnetosphere, should play the part of the trigger mechanism.

The observed source projects on to the Large Magellanic Cloud. Moreover, it lies close in position to the supernova remnant N49 in the Large Magellanic Cloud[2]. However, its energetics suggest that it is not only located in our Galaxy, but even comparatively close to the Sun. Indeed, if the source were at 55 kpc the luminosity of isotropic radiation in the initial impulse would be $\sim 5\times10^{44}$ erg s^{-1}, and that at the pulsating stage, $\sim 3.6\times10^{42}$ erg s^{-1}. Accordingly, the energy released in the initial impulse would be $\sim 1.2\times10^{44}$ erg, that at pulsating stage (lasting $\sim$100 s) $\sim 3.6\times10^{44}$ erg, and in line radiation, $\sim 10^{42}$ erg. The total energy in the event would then be $>4.6\times10^{44}$ erg. The shape of pulsations (Fig. 2) shows that the angular pattern of emission cannot be very narrow. Taking into account possible directivity of emission could not reduce these estimates by more than a factor of 10. Such large figures for the total energy and luminosity apparently rule out the possibility that this source is located in the Large Magellanic Cloud. On the other hand, if its average luminosity in the pulsating phase is close to that of X-ray sources in binaries (10^{37}–10^{38} erg s^{-1}), an estimated distance to it is 100–300 pc.

Consider some implications of the assumption on the galactic nature of this unusual X-ray pulsar. In its characteristics, mainly in the time scale of its variability and the presence of an impulsive initial stage, the FXP0520−66 source differs

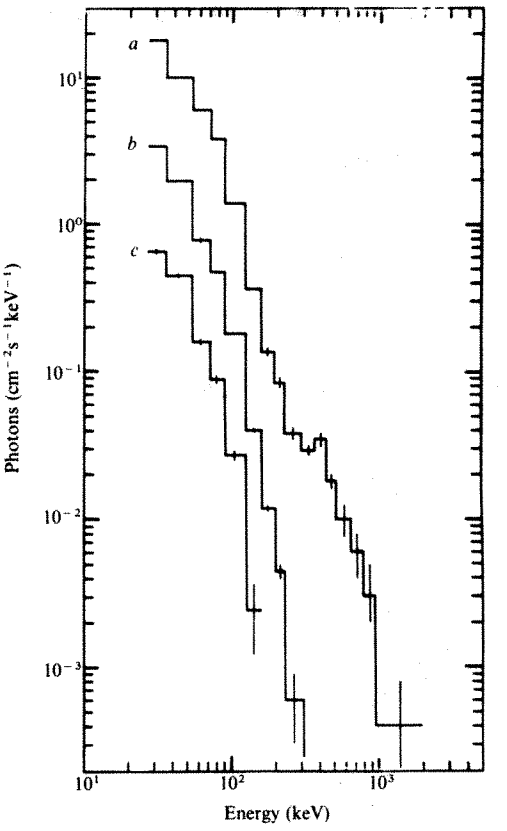


Fig. 3 Burst energy spectra. *a*, 5 March, spectrum of initial stage for the first 4 s; *b*, 5 March, averaged spectrum of pulsed radiation; *c*, spectrum of 6 March burst. Data from Venera 11 and Venera 12 are identical.

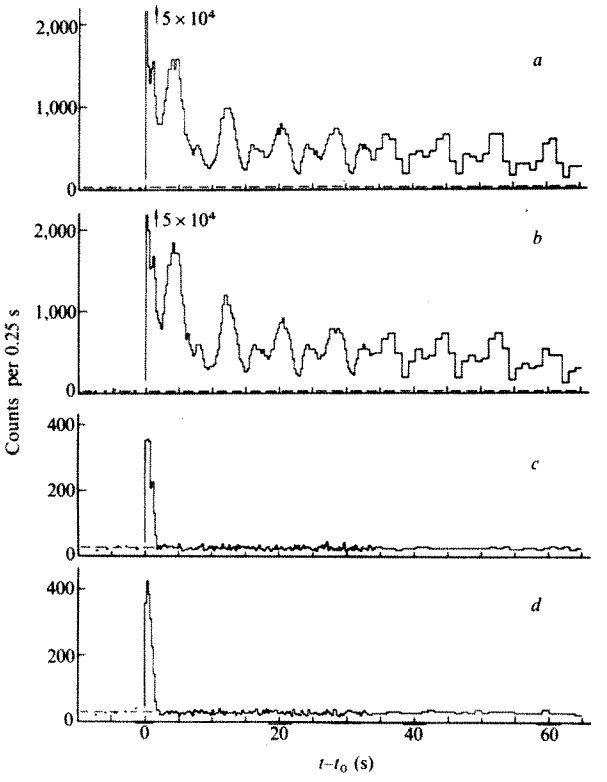


Fig. 2 Time structure of the bursts with resolution 1/4 and 1 s in the energy range 50–150 keV. *a*, 5 March, Venera 12; *b*, 5 March, Venera 11; *c*, 6 March, Venera 12: t_0 = 6 h 17 min 30 s, 455 UT. *d*, 6 March, Venera 11: t_0 = 6 h 17 min 25 s, 200 UT. Dashed line indicates background count rate. Points before t_0 show previous history of the bursts.

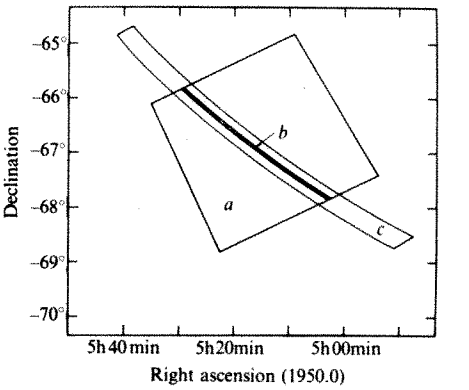


Fig. 4 Location of FXP 0520−66 source. Error box: *a*, for 5 March event, determined from detector anisotropic angular response; *b*, for 5 March event, obtained from detector anglular response and time-of-arrival difference; *c*, same for 6 March event.

markedly from the known transient sources[5]. It is unlikely that this source is the only one in the Galaxy. Other such sources probably exist and should be observable. However, the distances to them shoud be, on average, much larger, about a few kpc. Therefore, the observable radiation flux from such sources should be small. This implies that flaring X-ray pulsars could be detected primarily in their initial impulsive phase as short bursts of hard X-ray radiation.

Do the abovementioned differences in the spectral characteristics indicate that a binary cannot be the origin of γ-ray bursts? Apparently, not. Indeed, the revealed concentration of the γ-burst source locations in the direction of the galactic centre implies that distances to them are large and should be, on average, a few kpc (ref. 4). The corresponding estimates of the average energy released in γ-ray burst sources, 10^{40}–10^{41} erg, yield a value of 10^{39}–10^{40} erg s^{-1} for the average luminosity. These values exceed by at least 2 orders of magnitude the luminosity of known binary X-ray sources, as well as that of the FXP0520−66 source in its pulsating stage. Therefore, it is natural to suggest that the hardness and the shape of the radiation spectrum are substantially dependent on accretion rate, and it is the very high power of non-stationary accretion that determines the typically hard energy spectra of the γ-ray bursts that approach the power law in shape. The presence in the 5 March event of a hard component in the energy spectrum of the powerful initial impulse strongly supports this assumption.

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X-ray observations of the 5 March 1979 γ-burst field

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On 5 March 1979, an extremely intense burst of hard X rays and γ rays was recorded by the nine interplanetary spacecraft of the burst sensor network and localised by time-of-flight determinations to a position coincident with the supernova remnant N49 in the Large Magellanic Cloud[1–3]. Several times, both before and after the γ-ray event, we observed this region of the sky with the soft X-ray imaging instruments aboard the Einstein Observatory. Coupled with optical plate material, the soft X-ray data are used here to place severe constraints on models for the origin of this remarkable transient phenomenon.

The γ-ray observations are reported in the preceding letter[2]. Briefly, the salient features of the event include an extremely high peak flux (1.5×10^{-3} erg (cm^2 s)$^{-1}$ above 30 keV), a rapid rise time ($\leq$0.25 ms, ref. 1), a decline to a flux level 10^{-2} times that of the peak within a second, followed by a slow (~100 s) decay characterised by 8.1-s pulsations, a soft γ-ray spectrum which during the initial burst shows evidence of a line feature near 430 keV, and a second much weaker burst 14 h later. The morphology, period and spectrum of the pulsed component are reminiscent of pulsating binary X-ray sources, such as Her X-1 and Cen X-3, which are thought to contain magnetised rotating neutron stars and to be powered by accretion from a normal companion star. The case for the presence of a neutron star in the system responsible for the γ-ray event is strengthened if one interprets the 430 keV emission line as redshifted electron–positron annihilation radiation. The implied gravitational field is just that found due to a $1M_\odot$ object with a radius of 10 km. The positional coincidence of the γ-ray event and a supernova remnant (SNR) might be cited as further evidence that a neutron star was involved; however, the luminosities implied by the association of this event with the Large Magellanic Cloud are quite large: $L_\gamma\sim5\times10^{44}$ erg s^{-1} at the burst peak and $L_\gamma\sim4\times10^{42}$ erg s^{-1} during the pulsing phase. In what follows, the X-ray data are presented and then used to set limits on possible interpretations for the origin of the event.

The X-ray observations were carried out as part of a complete soft X-ray survey of the Large Magellanic Cloud that has been underway since the beginning of the year[4]. The instrumentation of the Einstein Observatory has been described elsewhere[5]. With the imaging proportional counter (IPC) in the focal plane of the telescope, a $1°\times1°$ field of view is imaged with a spatial resolution of 1.5′ (FWHM), while the spatial resolution obtainable with the high resolution imager (HRI) over a $25'\times25'$ field is 4″. The initial IPC pointing, which occurred 8 days before the burst, revealed the presence of two bright sources of soft X-ray emission centred on N49 and (N49), emission nebulae in the Large Magellanic Cloud previously identified as SNRs[6]. With the moderate resolution of the IPC, N49 was unresolved; its companion 6′ to the north-west showed some evidence of extended structure. The results of the subsequent HRI observations are shown in Fig. 1. The source associated with (N49) is seen to be a uniform, symmetric emission region with a diameter of ~40 pc. By contrast, N49 is ~25 pc in diameter with a strong enhancement in the south-east quadrant and bright knots of emission similar to those observed by Dopita and Mathewson[7] and D. Clark (personal communication) in recent maps of coronal [Fe XIV] emission. A detailed discussion of the X-ray structure of SNR in the Large Magellanic Cloud will be given elsewhere. Here, we concentrate on those aspects of the data relevant to the 5 March event.

Our first important constraint on models for the γ-ray burst derives from the constancy of the flux from the N49 region in the two IPC observations carried out 0.72×10^6 s before and 3.29×10^6 s after the γ-ray event. We can quantify this constancy, independent of instrumental or background effects, by taking the ratio of the flux in the region of N49 to that from (N49). These ratios in the two IPC images are 2.167 ± 0.09 and 2.185 ± 0.09, corresponding to a limit on the change in flux from N49 of $<0.8\%^{+2.9\%}_{-0.8\%}$. Assuming a power law spectral index of −2 and a low energy cutoff of 0.5 keV, this corresponds to a 3σ upper limit on the change in flux of 2.2×10^{-12} erg (cm^2 s)$^{-1}$.

The improved spatial resolution of the HRI permits us to derive a second result—namely, a limit on the strength of any point source within the instrument's $25'\times25'$ field of view. This limit is obtained by summing the number of counts detected in $8''\times8''$ regions of the map and then subtracting a local background counting rate determined from the eight adjacent regions. The limits thus obtained depend on the mean surface brightness of the area being searched. For the region of extended emission within N49, the 3σ upper limit is 9.9×10^{-3} c.p.s., whereas for the area outside N49 but still near the γ-ray error box, the limit is $\sim10^{-3}$ c.p.s. These point source limits correspond respectively to flux limits of 2.1×10^{-12} erg (cm^2 s)$^{-1}$ and 2.2×10^{-13} erg (cm^2 s)$^{-1}$ (0.5–4.5 keV). The greater of these two values is therefore essentially the same as the limit to any change in flux derived from the IPC.

These X-ray limits imply that, independent of distance, the steady-state (or remnant) luminosity from the system which produced the γ-ray event is less than 10^{-9} that observed above 30 keV in the burst. This translates to a luminosity limit of $2\times10^{30}\,d_{100}^2$ erg s^{-1}, where d_{100} is the distance to the source in units of 100 pc. At the Large Magellanic Cloud, the limit on L_x is $<4\times10^{35}$ erg s^{-1}, two orders of magnitude smaller than that of a typical pulsating binary X-ray source.

In Table 1, we present these limits along with the γ-ray luminosities and energies and a variety of model-dependent

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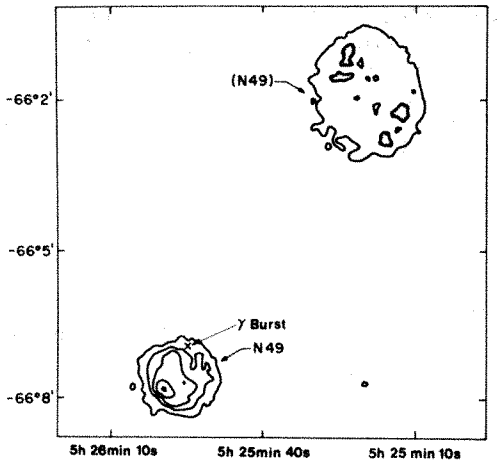


Fig. 1 Surface brightness contour map of the region containing N49 and (N49) as observed with the HRI. The map has been smoothed by convolving the raw data with a gaussian with a σ of 3″. The contour levels correspond to 0.025, 0.10, 0.20, 0.40 and 0.62 counts $[(1'\times 1')\,s]^{-1}$. The lowest contour ($\sim 3\sigma$ above the mean background level) corresponds to an equivalent flux of approximately 10^{-6} erg $(cm^2\,s\,keV)^{-1}$ in the energy range 0.5–4.5 keV. The position of the γ-ray burst (ref. 1) is shown; preliminary errors on this position are ~1.25′ in right ascension and 1.5′ in declination.

constraints implied by the observations. The sample distances are for a very nearby object (1 pc; see below), 200 pc suggested by Mazets *et al.*[2], 2 kpc corresponding to the limit for a galactic object in this direction ($|z|<1$ kpc), and 55 kpc, the distance of the Large Magellanic Cloud[8]. The neutron star temperature limits T_{ns} have been derived by convolving the radiation expected from an isotropically emitting black body of radius 10 km with the transmission characteristics of the interstellar medium (see ref. 9 for details). The masses of material involved in burst I on 5 March and burst II the following day, m_I and m_{II}, have been derived from the expression

$$m = \frac{E_\gamma}{c^2}\frac{1}{\eta_b} \qquad (1)$$

The results scale linearly with η_b, the efficiency with which the rest mass energy is converted to γ rays above 30 keV; as an example we have adopted a value of $\eta_b = 0.03$ appropriate for the conversion of hydrogen to helium in a thermonuclear flash. An interim mass accretion rate $\dot{m}_{I-II}$ is implied if the mass involved in burst II was accumulated between the events, while an upper limit to the steady-state accretion rate $\dot{m}_x$ can be derived from the X-ray observations:

$$\dot{m}_x < \frac{L_x}{c^2}\frac{1}{\eta_x} \qquad (2)$$

where the standard value for the conversion of accreted material to X rays in a typical X-ray binary system of $\eta_x = 0.1$ has been used. These two values imply that the accretion rate is highly variable, since

$$\frac{\dot{m}_{I-II}}{\dot{m}_x} > 200\frac{\eta_x}{\eta_b} \qquad (3)$$

Finally, the spectral type of any normal star associated with the system responsible for the burst is constrained by the fact that there is no star brighter than $m_B \sim 14.5$ on the ESO sky survey blue plate near or within the γ-ray error box; this observation rules out all but late-type, main sequence companions for the galactic distances and supergiant companions in the Large Magellanic Cloud.

Having tabulated these results, we consider the probable nature and distance of the system which produced the γ-ray event.

The remarkable positional coincidence of the γ-ray burst and N49 shown in Fig. 1 is the strongest argument for locating the system responsible in the Large Magellanic Cloud. Mazets *et al.*[2] reject this hypothesis because of the luminosities it implies. However, their implicit application of Eddington-limited accretion to the luminosity during the pulsation phase is clearly not required for such nonsteady flow. For example, the observed pulsed luminosity of 4×10^{42} erg s^{-1} (for a 55-kpc distance) is consistent with the thermal emission expected from a hot polar region with the observed temperature of 30 keV covering 10% of a neutron star surface. While current opinion seems to favour a galactic origin for γ-ray bursts[10], their spatial distribution is isotropic and consistent with an extragalactic origin. The fact that this burst was extremely bright may simply reflect its location in our nearest extragalactic neighbour, the Large Magellanic Cloud. However, this argument encounters severe difficulties if we assume that, despite some spectral and temporal differences, this γ-ray event is a manifestation of the same underlying phenomenon which produces all γ-ray bursts. The 5 March event was a factor of 10^2–10^3 more intense than other observed bursts. For an intrinsic spread in burst luminosities of an order of magnitude, this implies that the event was between 3 and 100 times closer than other bursts. An origin in the Large Magellanic Cloud, then, implies typical distances of 0.2 to 5 Mpc

Table 1 γ-Ray burst parameters

Parameter	Distance (pc)			
	1	200	2,000	55,000
$L_{\gamma I}$ (peak) (erg s^{-1})	2×10^{35}	7×10^{39}	7×10^{41}	5×10^{44}
$L_{\gamma I}$ (pulsed) (erg s^{-1})	1×10^{33}	5×10^{37}	5×10^{39}	4×10^{42}
$E_{\gamma I}$ (erg)	1.5×10^{35}	6×10^{39}	6×10^{41}	4.5×10^{44}
$E_{\gamma II}$ (erg)	1.5×10^{33}	6×10^{37}	6×10^{39}	4.6×10^{42}
L_x and ΔL_x (erg s^{-1})	$<1\times 10^{26}$	$<5\times 10^{30}$	$<5\times 10^{32}$	$<3.5\times 10^{35}$
T^*_{ns} (K)	$<1\times 10^{5}$	$<3.5\times 10^{5}$	$<9\times 10^{5}$	$<3.5\times 10^{6}$
m_I (g)	6×10^{15}	2×10^{20}	2×10^{22}	1.5×10^{25}
m_{II} (g)	6×10^{13}	2×10^{18}	2×10^{20}	1.5×10^{23}
$\dot{m}_{I-II}$ (g s^{-1})	1×10^{9}	4×10^{13}	4×10^{15}	3×10^{18}
$\dot{m}_x$ (g s^{-1})	$<1.5\times 10^{6}$	$<6\times 10^{10}$	$<6\times 10^{12}$	$<4\times 10^{15}$
Companions allowed	None	Dwarfs and subdwarfs later than K5; white dwarfs	Dwarfs and subdwarfs later than FO; white dwarfs	All but supergiants

* Values of 1 cm^{-3} for the galactic interstellar hydrogen density and 10^{21} cm^{-2} for the total column density to the Large Magellanic Cloud have been adopted.

which represent a volume of space containing too few galaxies to produce an isotropic burst distribution. Thus, starting from the Large Magellanic Cloud hypothesis, we are forced to conclude that either this burst represents a distinct class of events as suggested by Cline *et al.*[3] with a different underlying mechanism, or that it was more than 10 times weaker than a typical burst implying mean intrinsic luminosities for this phenomenon of $>10^{46}$ erg s^{-1}.

The same distance scaling factors (3–100 times closer) also apply to a galactic hypothesis. For a scale height of 1 kpc which is consistent with the observed burst distribution[10], this event must have been located between 10 and 300 pc from the Earth. Models for the origin of such a nearby event are constrained by the various limits presented in Table 1. Neutron star temperature limits of a few hundred thousand degrees are only marginally consistent with those expected from undisturbed, isolated objects[9]. In addition, the various accretion rates coupled with the limits on an optical companion can be used to constrain the parameters of models containing binary systems.

By using the observation of the narrow 430-keV emission feature observed in the burst spectrum, we can derive another estimate of the source distance. If this feature is real, an upper limit to the distance of the neutron star may be obtained if we assume that the continuum emission arises from electron bremsstrahlung and that the emission region (assumed for simplicity to be spherical and of uniform density) is the same for both the line and the continuum. For a narrow line feature to exist, the electron scattering depth must not greatly exceed unity; that is,

$$\sigma n_e r = \tau_{es} \lesssim 1 \tag{4}$$

where σ is the electron scattering cross-section at 430 keV, n_e is the ambient electron density and r is the radius of the emission region. The distance of the event determined from the continuum radiation is given by

$$d = 3^{-1/2} F^{-1/2} \varepsilon^{1/2} n_e r^{3/2} \tag{5}$$

where F is the continuum flux and $\varepsilon (\sim 1.64 \times 10^{-27} T^{1/2}$ erg cm^{-3}) is the volume emissivity of a gas with unit density. Thus, in units appropriate if the positron line were present only during the initial fast part of the burst and assuming that the light travel time of this portion of the event sets a limit on the size of the emitting region, we have

$$d \lesssim 2.3 \times 10^{18} \left(\frac{1.5 \times 10^{-3} \text{ erg (cm}^2 \text{ s)}^{-1}}{F} \right)^{1/2} \times \left(\frac{T}{30 \text{ keV}} \right)^{1/4} \tau_{es} \left(\frac{t}{10^{-3} \text{ s}} \right)^{1/2} \text{cm} \tag{6}$$

which is <1 pc! Unfortunately, the time resolution of the spectra presented by Mazets *et al.*[2] is 4 s. If values for the continuum flux are determined over this interval and the diameter of the emitting volume is assumed to be 4 light s, the upper limit is still less than 100 pc. The closest distances are probably excluded, in that X rays from interstellar accretion onto an isolated neutron star would violate our point source flux limits. We note that for $d \lesssim 10$ pc, however, such an isolated star can accumulate sufficient interstellar material to satisfy the entire γ-ray burst energy requirements in <10 years.

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Solar structure from global studies of the 5-minute oscillation

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High sensitivity Doppler spectroscopy of integral sunlight in the 769.9 nm line of neutral potassium reveals several equally spaced lines of $Q > 1{,}000$ centred on the well known period of 5 min. These lines are interpreted as low l, high n overtones of the entire Sun and, if viewed in terms of current solar models, appear to imply a low heavy element abundance and a consequent low solar neutrino flux. Previous workers[1–3] have used spatial resolution to study the 5-min oscillation of the solar photosphere and have shown that power is concentrated along narrow ridges in the k, ω diagram. These oscillations are mainly low n, high l value acoustic modes of the entire Sun. In contrast to this, the present work (for a summary see ref. 4) is aimed at an overall view of the solar surface and consequently is biased to a study of low l value oscillations. These modes penetrate deeply[5] and thus probe the structure as well as the interior angular velocity of the Sun[6].

Doppler shift measurements of integral solar light show several discrete lines of amplitude 0.1–0.3 m s^{-1} within the main peak of the power spectrum of the 5-min oscillation. These lines are found to have, on average, a uniform spacing of 67.8 μHz.

If the lines are interpreted as being due to low l value oscillations of the entire Sun, then the observed spacing taken in the context of current solar models[7,8] implies a mean heavy element abundance of $Z \sim 0.004$ and a consequent neutrino flux of ~2.3 SNU, within two standard deviations of the experimental result[9].

Line of sight velocity measurements of the whole solar disk were made using optical resonance spectroscopy, a technique

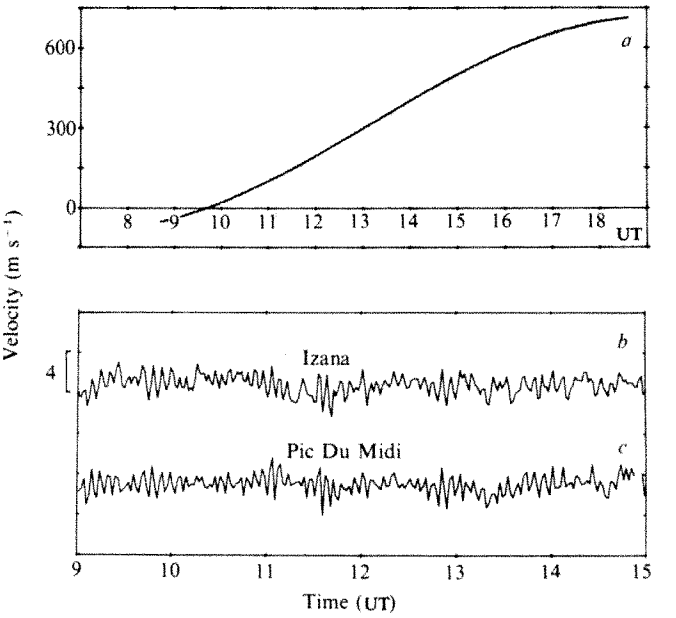


Fig. 1 Mean line of sight velocity observed on 6 August 1978 at Izana (*a*). Comparison of residuals obtained simultaneously at Izana (*b*) and Pic du Midi (*c*).

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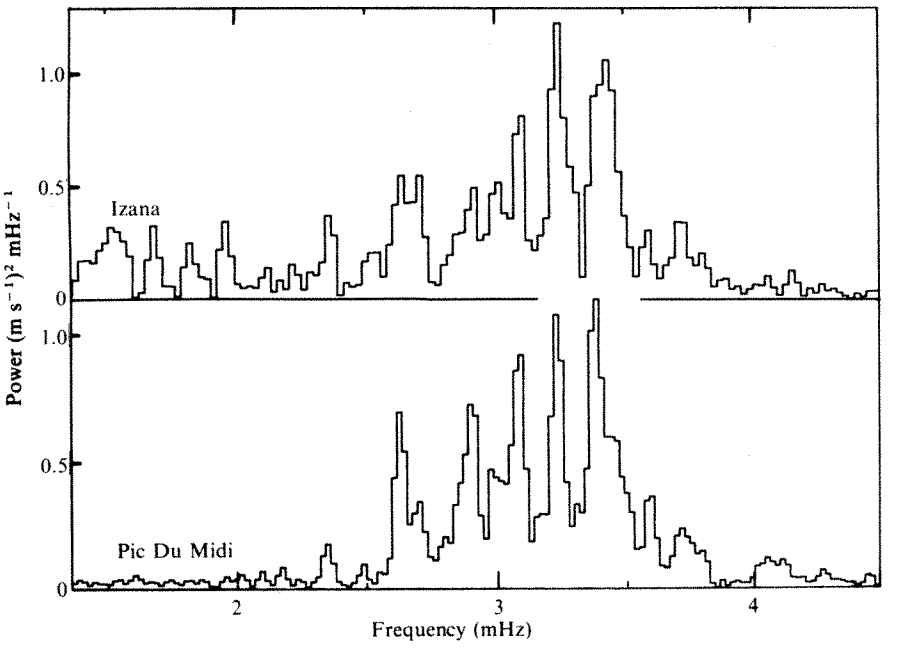


Fig. 2 Power spectra of the residuals obtained from data taken on 6 August at Izana and Pic du Midi.

which has been described in detail previously[10]. Briefly, the velocity of the solar photosphere with respect to the laboratory is measured by comparing the position of the Fraunhofer absorption line of neutral potassium at 769.9 nm with that of the same line in the laboratory. Observations were made during 1976, 1977 and 1978 at Izana, Tenerife and in 1978 simultaneously at Pic du Midi in the Pyrenees. The two sites are ~2,300 km apart and are expected to be meteorologically uncorrelated, thus allowing solar effects to be distinguished from atmospheric or instrumental perturbations. In the 1977 and 1978 seasons the spectrometer incorporated substantial improvements with respect to guidance, optical components, thermal stability and statistical accuracy, resulting in the collection of data of improved quality.

The Sun was observed for about 8 h on each of 33 days in 1976 (a total of 269 h) and 35 days in 1977 (total 280 h). These data as well as those from 7 of the days obtained in 1978 have been analysed. The data for any one day consist of a mean line of sight velocity determined every 42 s (100 s on Pic du Midi) for the whole observing day (Fig. 1*a*). Residuals corresponding to velocity amplitudes of less than 3 ms^{-1} (Fig. 1*b*) show up after subtraction of the observer's velocity relative to the centroid of the Sun due to the spin and orbital velocities of the Earth. A small allowance for residual curvature effects has been made.

Residuals of data obtained simultaneously at the other observing site are shown for comparison in Fig. 1*c*. To compare these data directly, those from Izana have been smoothed by a moving mean over three points (126 s) and then summed over two points (84 s) to simulate the 100-s integration time of the Pic du Midi data. The correlation of these two data strings clearly demonstrates that the observed signals can only be attributed to a solar origin.

These residuals form a time series which may be processed using standard power spectrum techniques[11]. The power spectra obtained for the data of Fig. 1*b* and *c* are shown in Fig. 2. The agreement of these two spectra, obtained simultaneously at two sites separated by 2,300 km, clearly reinforces the statement that the observed oscillations are of solar origin. A series of well defined peaks can also be seen.

To investigate these peaks further the power spectra for individual days were averaged for the 1976, 1977 and 1978 data. These spectra are shown in Fig. 3. The structure is clearly present and an apparent constancy in the spacing of these peaks can be seen. To verify that this spacing is not an artefact of the analysis, different lengths of data strings were used but this had no effect on the observed spacing.

The constancy of the peak spacing may be demonstrated in two ways. First, the observed peaks in the power spectra are numbered sequentially and plots of the order of the peak against the observed frequency are shown in Fig. 4*a* for the 3 yr of data. Where the line spacing clearly suggests that a particular line is absent in the experimental data, the order of the line has been

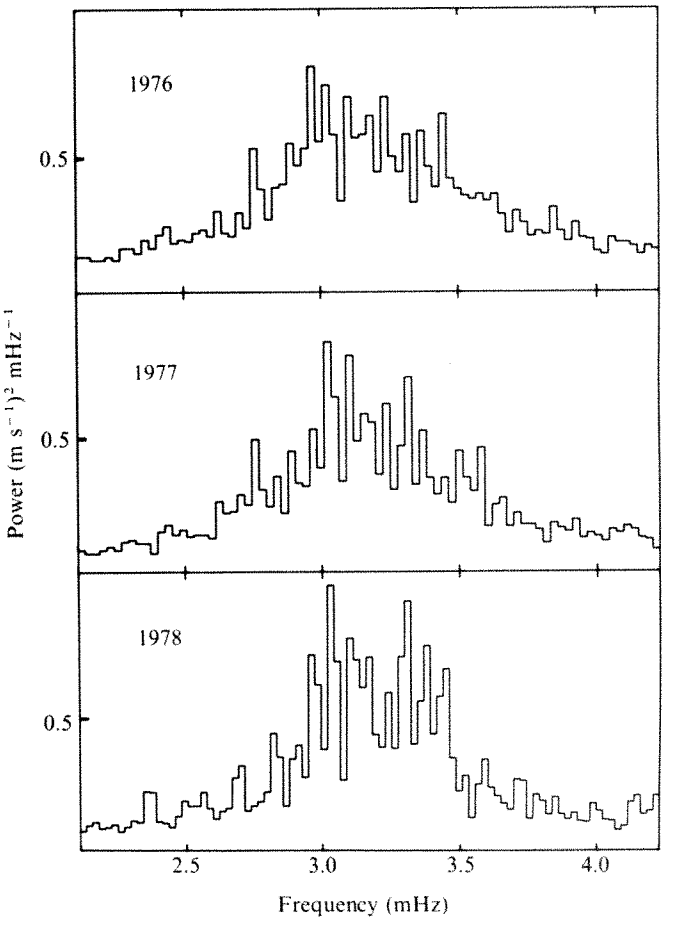


Fig. 3 Mean power spectra obtained from data collected over 33 days in 1976, 35 days in 1977 and 7 days in 1978 at Izana.

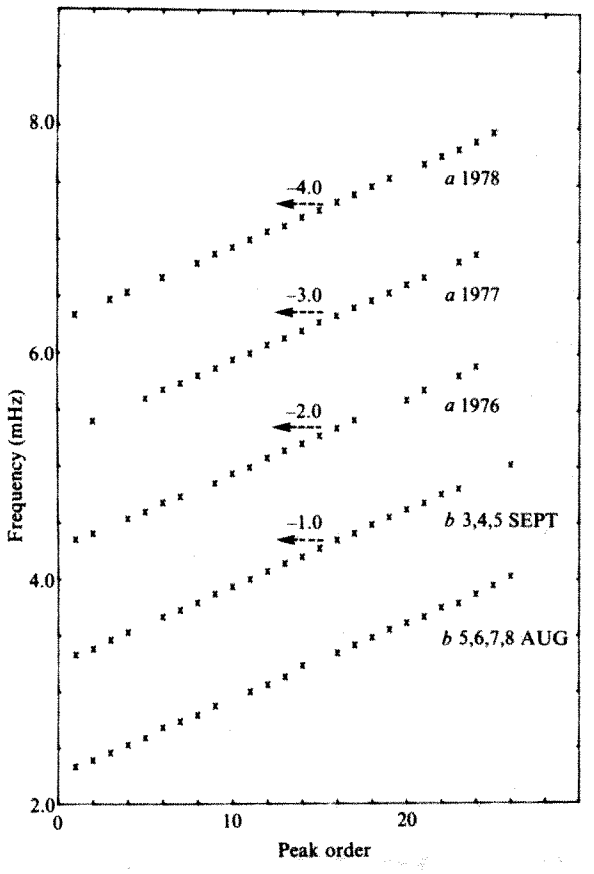


Fig. 4 *a*, Peak frequency against order plotted for the mean power spectra of 1976, 1977 and 1978. Successive plots are displaced by 1 mHz on the vertical scale as indicated. *b*, Similar plots for data determined by a superimposed epoch analysis for 4 days in August and 3 days in September 1978.

appropriately increased. The data are well fitted by straight lines and yield mean spacings of 67.6, 67.4 and 67.6 μHz for the 3 yr. Second, the mean power spectra data are screened by a high pass filter by subtracting a moving mean over three points, and then subjecting the resulting points to an autocorrelation and power spectrum analysis. In this way mean line spacings of 67.8, 68.0 and 68.0 μHz are found for the 3 yr. A cross-correlation analysis of the 1976 and 1977 power spectra yields a correlation coefficient of 0.89 and has a maximum at zero frequency lag. This firmly establishes the consistency of the lines over the 2 yr.

As an alternative form of treatment, the data of 1978, which are of higher quality due to improvements in experimental technique, were subjected to a superimposed epoch analysis. Four consecutive days in early August and three in September were used for this study, the frequency range being restricted to that covered by the power spectrum analysis. The structure found in the power spectrum analysis was again evident and a plot of the frequency against peak order is shown in Fig. 4*b*. Straight line fits to these plots yielded mean line spacings of 67.4 ± 0.5 and 67.9 ± 0.2 μHz, respectively. The results of the superimposed epoch analysis over the four consecutive days seem to imply that the Q value of these oscillations is $>1{,}000$.

To investigate further the coherence of the oscillation, a power spectrum analysis of two consecutive days of data was undertaken with zeros in the data string corresponding to the times when actual data were not available. This resulted in a 32-h data string whose power spectrum is shown in Fig. 5*a*. The usual peaks in the power spectrum are clearly evident. To test for the coherence of these peaks sine waves of the indicated frequencies were subtracted from the original data by using a least squares fitting procedure to optimise the frequency, phase and amplitude of the fitted wave. The new power spectrum obtained after subtraction of these coherent fitted sine waves from the data is shown in Fig. 5*b*. This clearly demonstrates that the oscillations are coherent over the 32-h data string and thus have a $Q > 400$.

It seems likely that these lines correspond to normal modes of vibration of the whole Sun. To identify these modes with certainty, information on their spatial structure is required. An integral light spectrometer tends to average out modes of high l value with many peaks and troughs across the visible disk of the Sun[6], thus strongly biasing the observations towards low l value modes. An approximate calculation of the sensitivity of a velocity spectrometer to various spherical harmonic modes can be found elsewhere[13] but a more precise evaluation must include the spectral weighting function across the solar disk[14]. The above suggests that the observed modes are of low l value, possibly $l = 0$ and $l = 1$, with even and odd l values alternating as the value of n (number of radial nodes) increases. The observed mean spacing of 135.6 ± 0.3 μHz of next nearest lines falls in the range of spacings (130–142 μHz) predicted by solar models with uniform, as well as surface enhanced, heavy element abundances. An extrapolation of the uniform model calculations[7]

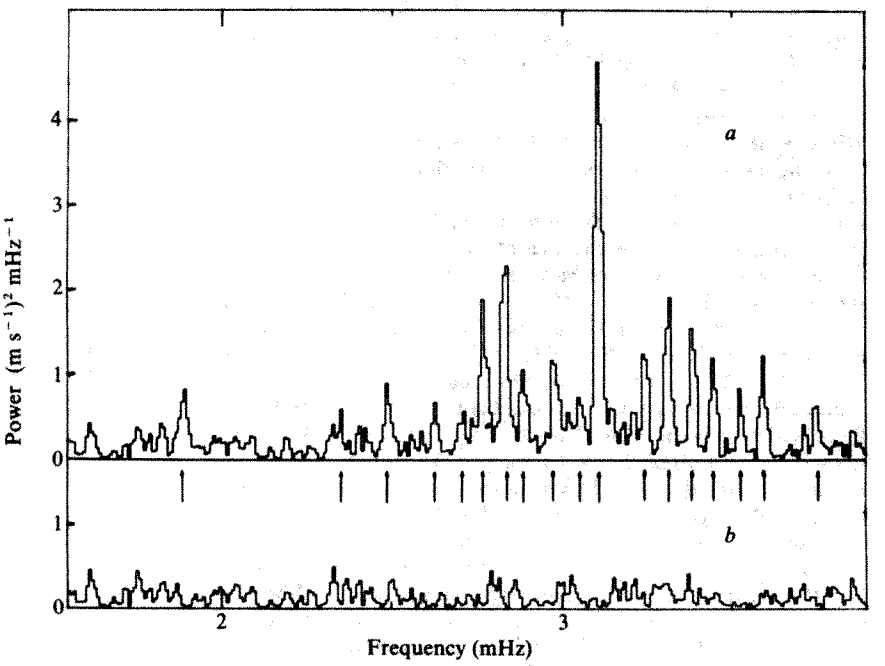


Fig. 5 *a*, Power spectra of two consecutive days of data obtained at Izana on 4 and 5 August 1978. *b*, Power spectra of the same data after subtraction of indicated discrete frequencies.

suggests that the observed spacing is consistent with a heavy element abundance of $Z \sim 0.002$. An interpolation of the non-uniform model predictions[8], with a surface heavy element abundance of 0.02, indicates that a mean heavy element abundance of $Z \sim 0.004$ closely fits the observed spacing. Assignment of the lines to l values ≥ 2 is either not consistent with the uniform model[7] or leads to even lower Z values for the non-uniform model[8]. This suggests that any interpretation in terms of these models implies a Z value ≤ 0.004 and a solar neutrino flux of ≤ 2.3 SNU, consistent with the observed value of 1.75 ± 0.4 SNU[9].

Values of n can be tentatively assigned to the lines using the uniform model for which frequencies of particular modes in this region[7] have been calculated. Using the low l value assignment and the approximate Z value limit quoted above, the observed frequencies of 2.41 and 4.04 mHz are tentatively identified as $l = 0$ modes with $n = 17$ and 29, respectively. Such an identification is consistent with the observed slight inequality in spacing between alternate lines. Thus, if the frequencies ν of two neighbouring $l = 0$ lines are compared with that of an $l = 1$ line, as expected[7], $\frac{1}{2}(\nu_{n+1,0} + \nu_{n,0}) > \nu_{\text{odd}}$. The heavy element abundance corresponding to this identification is near $Z = 0.004$.

The discrete lines are expected to be hitherto unresolved multiplets due to incomplete degeneracy of different n, l contributions. Rotation of the Sun is further expected to split the modes into $(2l + 1)$ components of spacing characterised by the mean angular velocity of the interior[6,14]. This will produce a long period amplitude modulation of the lines, some evidence for which already exists. It should be possible to resolve this fine structure by using long data strings, provided that the coherence time is adequate.

The definite assignment of the various modes is being attempted by a two-dimensional study of the solar photosphere. The amplitude variation of particular lines, as well as phase information, is being analysed further to study the excitation and damping mechanisms.

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Spurious radiation from microwave ovens

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In agreement with the regulations of the International Telecommunication Union the manufacturers of microwave ovens are licensed to operate within the frequency band 2.45 ± 0.05 GHz reserved for industrial, scientific and medical purposes. Evidence that microwave ovens emitted significant amounts of radiation outside this licensed band and covering the reserved bands allocated to radioastronomy and communications was given in a report prepared for the Royal Society and Ministry of Technology in 1969[1]. No action was taken by the licensing authorities to enforce restrictions on this spurious radiation and because the use of microwave ovens has become widespread we have made and report here a new series of measurements to assess the extent of this radiation from the ovens now in use. Our main concern has been with the spread of the oven radiation into four of the bands protected internationally for the use of radioastronomers: 1,400–1,427 MHz (21 cm H-line band); 1,660–1,670 MHz (18 cm OH band); 2,690–2,700 MHz (11 cm S-band); 4,990–5,000 MHz (6 cm C band). Although our primary interest has been to estimate the limitations which may be placed on the performance of the radio telescopes at Jodrell Bank by microwave ovens used in the vicinity, the results are relevant to the communication systems that are allocated nearby frequency bands.

Measurements have been made on three ovens from different manufacturers; two were new domestic models currently on sale and the third was a larger commercial model that had been in service for 10 years and was coated with deposits of grease and grime. As it is unwise to operate the ovens without a load, a beaker containing approximately 300 ml of water was placed centrally on the shelf of the oven. The radiation levels were measured with a tunable receiver connected to a log-periodic antenna having nearly constant gain over the band 1–6 GHz. The receiver sensitivity was measured by means of a calibrated signal generator.

It was established that the received signal did not depend significantly on the polarisation of the antenna. The data presented here were obtained with the antenna vertically polarised and directed at the front of the oven from a distance of 3 m. Figure 1 shows an oscilloscope display of the total power received by the antenna in a bandwidth of several gigahertz (*b*), and the power received in a 7 MHz bandwidth centred on 2,695 MHz (*a*). The oscilloscope time base was triggered at a rate of 50 Hz; the exposure time was 1 s. The rotation of the

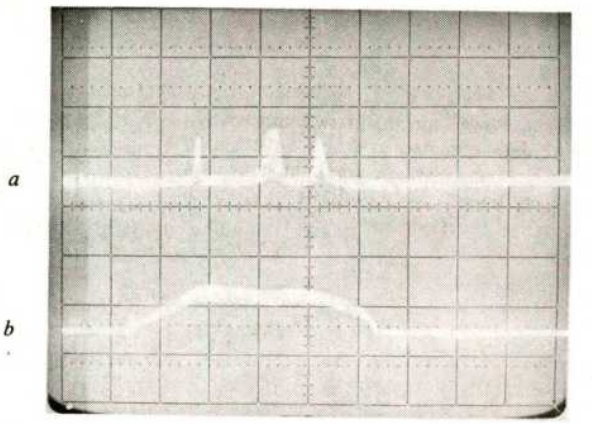


Fig. 1 The power received by the antenna as a function of time. *a*, The output of a receiver tuned to 2,695 MHz with a bandwidth of 7 MHz. *b*, The total power received by the antenna. The time base was triggered at a 50 Hz rate and the total time displayed is 20 ms.

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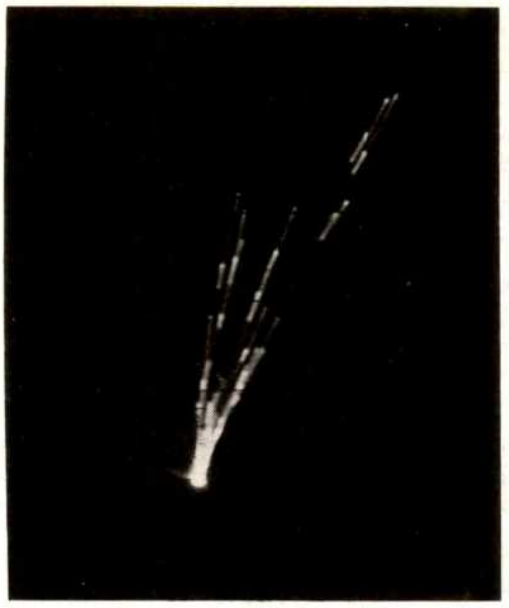


Fig. 2 A polar display of the instantaneous frequency of the major component received by the antenna. The radial distance from the bright spot is a measure of the intensity and the azimuthal angle is a measure of the frequency. One rotation covers 88 MHz.

metal paddle inside the oven was inhibited during the exposures. Movement of the paddle by hand affected the amplitude of the lower trace and caused the position of the short pulses on the top trace to move back and forth in time. Tuning the receiver centre frequency over tens of MHz had the same effect on the short pulses.

Essentially the same behaviour was observed wherever the receiver was tuned between 1 and 6 GHz except that the top trace became similar to the bottom trace near and below the nominal oven operating frequency of 2.45 GHz and also near the second harmonic at 4.9 GHz. With the paddle stationary, it was possible to find restricted regions of the spectrum where the radiation was below our limit of detection in a 7 MHz bandwidth but these quiet regions were much less frequent if the paddle was rotating.

Figure 2 shows a polar display of the total power (radial distance) versus instantaneous frequency (position angle of the trace with respect to the bright central spot) received by the antenna. A 90° difference in position angle corresponds to a frequency difference of 22 MHz. Each 'finger' on the photograph is a mode of the oven and the trace intensity is proportional to the probability of finding the output at that power and frequency. The largest observed separation of the modes was ~30 MHz. It seems, therefore, that the major components of emission fall within the frequency limits specified.

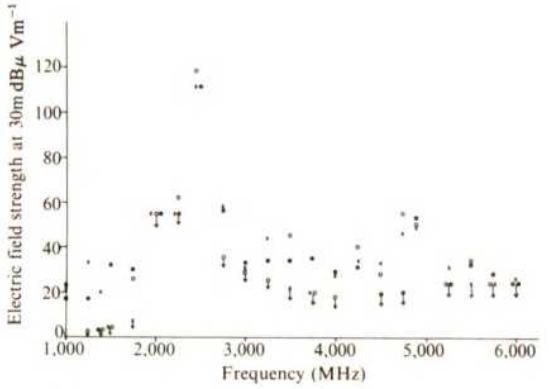


Fig. 3 Electric field strength at a distance of 30 m as a function of frequency for the radiation from three microwave ovens: ○, used commercial oven; × and ●, new domestic ovens.

Figure 3 shows the peak value of the electric field components of the spurious emissions from the ovens measured in a 30 kHz bandwidth and normalised to a range of 30 m (assuming an inverse square relationship for power). The measurements are plotted for all three ovens at 0.25 GHz intervals between 1 and 6 GHz. These data are in good agreement with earlier Post Office measurements of a completely different set of ovens manufactured between 1962 and 1968 (ref. 1).

The interference effects from microwave ovens on radio telescopes working in the frequency bands allocated for astronomical purposes and listed above have been calculated as follows. The receiving system on a telescope generates a noise signal, P_N, in the receiver bandwidth, B, equivalent to a mean input power given by

$$P_N = kT_sB\,\mathrm{W}$$

where k is Boltzmann's constant and T_s is the effective system noise temperature in degrees Kelvin. If the receiver output is averaged for a time τ, the input power can be established with a statistical uncertainty

$$\delta P_N = kT_s(B/2\tau)^{1/2}$$

Signals from radio sources that add more power to the receiver than δP_N can be detected with a statistical confidence dependent on the factor by which the added power exceed δP_N in the usual way for a normal error distribution.

Table 1 Specification of the maximum levels of radiation in the 1 to 6 GHz band from interfering sources assuming T_s is 30 K

A Mean power from interfering source	$<2\times10^{-20}$ W in 10 MHz bandwidth $<0{\cdot}65\times10^{-21}$ W in 10 kHz bandwidth $<0{\cdot}65\times10^{-22}$ W in 100 Hz bandwidth	assuming $\tau = 2\times10^{3}$ s	
B Peak power averaged over 1 ms	$<2.8\times10^{-17}$ W in 10 MHz bandwidth		($\delta t = 10^{-3}$ s)
C Peak power averaged over 1 ms of a repetitive signal with period > several milliseconds	$<2.8\times10^{-19}$ W in 10 MHz bandwidth		($N\delta t = 10$ s)

Many astronomical radio sources have continuous spectra so that the telescope receives from the source a power proportional to the receiver bandwidth. A larger value of B then increases the signal-to-noise ratio despite the proportionality of δP_N to $(B)^{1/2}$. A long integration time also improves the signal-to-noise ratio for those sources for which the intensity is not a function of time. In the case of pulsars where the intensity of the source is a function of time, τ is limited to the desired time resolution, δt, if the purpose is to study the structure of a single pulse, or to $N\delta t$ if the purpose is to study the integrated profile of N pulses with time resolution δt.

The power received from interfering sources is not generally constant as a function of time: either the source emission varies or the telescope sweeps its sidelobe structure over the source as it tracks an astronomical object. The net result in either case is to increase the fluctuations in the receiver output above the natural limit and hence impair the sensitivity of the radio telescope. The power received from interfering sources must be below δP_N if the performance of the telescope is not to be affected.

On this basis, limits may be placed on the tolerable power levels of interference for a radio telescope. These limits are given in Table 1 assuming that the system noise temperature, T_s, is 30 K. It must be stressed that Table 1 is based on assumptions and limits which are typical of contemporary systems used in radioastronomy and are, in fact, 10 times less severe than the

CCIR Report on the protection criteria for radioastronomy[2]. On the other hand, we assume that there is only one source of interference which is not likely to be the case if microwave ovens become widespread.

The power (P_i) received from an interfering source by a radio telescope depends on the equivalent isotropically radiated power (EIRP) of the source in the direction of the telescope, the range of the source from the telescope (R_i), and the gain of the telescope (G_i) in the direction of the source. The relationship between these parameters is

$$P_i = (EIRP)G_i(\lambda_i/4\pi R_i)^2$$

where λ_i is the wavelength of the radiation. The appropriate G_i to use in the case of a ground-based source of interference is that of a far-out sidelobe of the telescope response for which $G_i \sim 1$ (a −63 dB sidelobe for a 80-m telescope at $\lambda \simeq 12$ cm).

Measurements were made on the interference from one of the domestic ovens in the protected frequency bands for radioastronomy shown in Table 1. The results are shown in Table 2 together with a comparison with limits A and C of Table 1. These results have been converted to levels received by a radio telescope at a range of 1 km assuming that G_i is 1 and that power flux density is $\propto R_i^{-2}$. Measurements were also made 50 MHz on either side of each of these bands as it was thought that there might be nulls in the spectrum that were oven-load dependent or not repeatable from oven to oven.

The data presented in Table 2 show that spurious emissions from microwave ovens present a serious danger to radioastronomical researches and at some parts of the spectrum they are at a level that would degrade the performance of a point-to-point radio link where the possibility of having an oven in the main beam of, and close to, a receiver cannot be discounted.

The power levels given in Table 2 for the four protected frequency bands have been used to derive the data given in Table 3 for the influence of a single oven operating at various distances from a radio telescope with a sidelobe level with a gain equivalent to an isotropic radiator. Table 3 makes no allowances for any screening of the oven that may be provided by the walls of buildings, or other obstructions. The Post Office report[1] estimates that up to 30 dB attenuation of the signals may be caused by such obstructions but this form of screening cannot be relied on—a glass window is relatively transparent to microwaves and an average kitchen window would allow spurious emissions from an oven to escape unattenuated over a wide range of angles.

The emission of significant microwave power over such a wide spectral range seems to arise from the method of generation of the power coupled with the ineffective shielding at the seals of the oven door. The source of the microwaves is a magnetron producing 1–2 kW of power and operating from rectified but unsmoothed a.c. mains. The power is generated for approximately half of each supply cycle. The magnetron drives a load that is deliberately disturbed by a rotating metal paddle in the oven with the consequence that both the instantaneous frequency at which it oscillates and the power that it delivers are functions of time. The instantaneous frequency lies in the assigned band of 2,450 ± 50 MHz but modulation sidebands exist and it is these which give the out-of-band emissions. It is also possible that some of the spurious emissions are caused by instabilities in the electron distribution inside the magnetron leading to the generation of unwanted frequencies[3,4].

The primary source of leakage of radiation is from the seals around the door which fail to confine the microwave radiation to the inside of the oven[5]. These seals are non-contacting and seem to consist of a resonant, quarter-wavelength choke nominally tuned to 2,450 MHz followed by microwave absorbing material. The seals are sufficiently effective in the assigned band of 2,450 MHz to satisfy the safety regulations appropriate to the UK[6], but they fail to give adequate out-of-band suppression to prevent possible interference with other services authorised to operate within the 1–6 GHz frequency band.

The measurements of the spurious emission from microwave ovens over the frequency band 1–6 GHz are in substantial agreement with those reported previously[1]. In particular these emissions cover at least four of the frequency bands which are allocated by the ITU to radioastronomy. Increasing use of the ovens in the vicinity of radio astronomical installations and of communication systems working in this frequency range would place serious limitations on the operation of these installations. Whereas the Radio Regulatory Department of the Home Office place stringent limits on the amount of the spurious radiation which is allowed from communication systems no such limits seem to be imposed on the amount of the out-of-band radiation allowed from microwave ovens.

The design of the ovens on sale in UK is governed by a directive[6] which recommends an upper limit for continuous exposure of 10 mW cm^{-2} and specifies that the test equipment should be capable of measuring to 1 mW cm^{-2} and preferably to 0.5 mW cm^{-2}. Furthermore it is specified that the equipment used for the measurements should be "relatively insensitive outside its declared frequency band". The measurements reported here reveal that this specification (laid down in 1960) is not sufficient. The regulations need revision so that the use of microwave ovens is not allowed if the amount of the emitted radiation outside the licensed band of 2,450 ± 50 MHz can cause harmful interference to authorised radio services.

Table 2 Levels of interference from a microwave oven at a distance of 1 km for radiation received in a far-out sidelobe ($G = 1$)

Frequency (MHz)	Mean power into receiver at 1 km, 10 Mhz BW (W)	Peak power into Rx at 1 km 10 MHz BW (W)	Factor by which mean exceeds limit A of Table 1	Factor by which peak exceeds limit C of Table 1
1,365	5×10^{-20}	$2{\cdot}5\times10^{-18}$	2.5	9
1,415	2.5×10^{-19}	5×10^{-18}	12.5	18
1,465	1.5×10^{-19}	7.5×10^{-18}	7.5	27
1,620	2.5×10^{-20}	2.5×10^{-18}	1.25	9
1,665	$<0.75\times10^{-20}$	$<2.5\times10^{-20}$	<0.4	<0.09
1,715	$<0.75\times10^{-20}$	$<2.5\times10^{-20}$	<0.4	<0.09
2,645	1×10^{-18}	1.5×10^{-16}	50	530
2,695	1.25×10^{-17}	2.5×10^{-15}	625	8,900
2,745	1.5×10^{-17}	2×10^{-15}	750	7,100
4,945	1.25×10^{-18}	2.5×10^{-17}	62.5	89
4,995	$\sim1.5\times10^{-20}$	$\sim1.5\times10^{-18}$	~0.75	5.4
5,045	$\sim0.5\times10^{-20}$	$\sim0.5\times10^{-18}$	~0.25	1.8

Table 3 Range of detectability as a function of frequency of the spurious emission from a microwave oven (line of sight without local screening)

Frequency (MHz)	Range in a sidelobe with $G_i = 1$ (km)	Frequency (MHz)	Range in a sidelobe with $G_i = 1$ (km)
1,365	1.6	2,645	7
1,415	3.5	2,695	25
1,465	2.7	2,745	27
1,620	1.1	4,945	8
1,665	<0.6	4,995	~0.8
1,715	<0.6	5,045	~0.5

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Corrosion micromorphology of noble metal alloys and depletion gilding

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Noble metal alloys commonly undergo selective dissolution during anodic corrosion whereby the less noble component is dissolved preferentially. This is particularly interesting, as it leads to a depletion of the less noble species not only at the surface of the metal but also at an appreciable depth within it, implying that a mass transport of atoms is occurring either through the bulk of the crystal lattice or across the surface to support the continuing dissolution. Experiments in which the micromorphology of silver–gold alloys is observed during such corrosive treatment have shown that surface diffusion of gold plays an important part in this process. As surface silver atoms are dissolved the residual gold atoms reform into gold-rich islands so that fresh silver atoms are continuously exposed to the environment layer by layer. These observations will be described in detail elsewhere[1]. Here the mechanism of surface re-arrangement is considered, which leads to the accumulation of gold-rich surface regions, and throws a new light on the ancient craft of depletion gilding.

Depletion gilding, or gold colouration (*mise-en-couleur*) is the process by which the surface of a dilute gold alloy becomes gold-enriched by the selective removal of the less noble component. This usually involves annealing the alloy or immersing it in a corrosive bath, or some combination of these treatments. It is well established that the Indians of pre-Columbian Central America had invented such a technique for the colouration of castings prepared from copper–gold alloy[2], commonly referred to as tumbago. Their colouring methods involved either the formation of copper oxide by heating in air, followed by chemical dissolution of the oxide or the slow removal of copper from the surface of the alloy in a corrosive bath. The 'spongy' gold layer so-formed was then consolidated by burnishing. It is thought that similar processes were used by North Peruvian craftsmen to colour objects fabricated from silver-based alloys[3]. However, the details of these processes, particularly the nature of the chemical reagents used, are still matters for conjecture.

A very closely related process for the colouration of silver–gold alloys, known as cementation, is known to have been used by European and Near Eastern goldsmiths since before the early Middle Ages. The object is packed in a reactive powder (invariably containing salt) which, when heated, forms soluble chlorides with silver and other impurities in the alloy, leaving the surface of the object gold-rich.

From the metallurgical point of view the most interesting aspect of these ancient crafts is the process by which the gold becomes parted from its alloys by the selective corrosion of the less noble components. Nitric acid has been used for parting silver and gold in Europe since the twelfth or thirteenth century. However, apart from the very superficial observation that nitric acid preferentially dissolves the silver, leaving the surface of the alloy coated with a spongy gold layer[4], there has been no detailed study of the metal physics of these surface enrichment processes. The micromorphological observations reported here may, however, provide some new insight into the understanding of these ancient technologies.

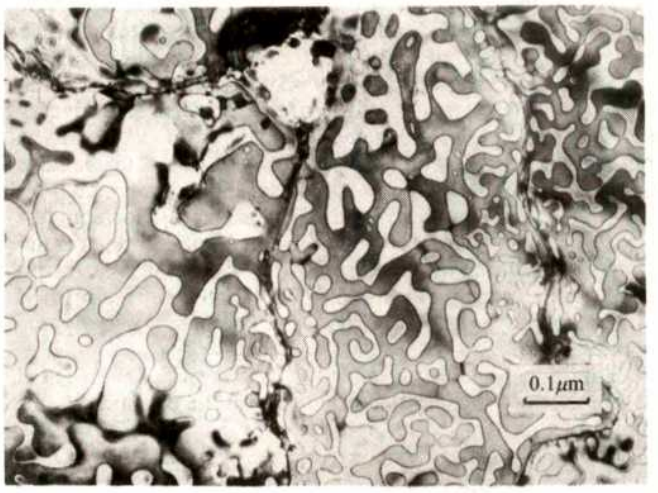


Fig. 1 Transmission electron micrograph of 50:50 Ag–Au alloy after corrosion in 50% nitric acid, showing island and channel morphology.

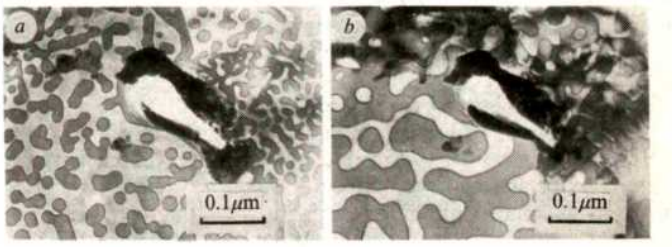


Fig. 2 Transmission electron micrograph of 50:50 Ag–Au alloy: *a*, after corrosion for 30 s in 50% nitric acid; *b* after corrosion for 60 s in 50% nitric acid.

Figure 1 shows a transmission electron micrograph of a single crystal thin film of a 50:50 silver–gold alloy after a small amount of corrosion in a 50% aqueous solution of nitric acid. The film, originally structureless, apart from a small number of dislocations, twins and double-positioning boundaries[5], has become transformed into a maze-like structure of island of more strongly absorbing material separated by more transparent channels. The selected area X-ray analysis facility available in the electron microscope, has shown that the island regions of the film are gold-rich compared with the channels, which is consistent with the suggestion that the islands are formed during corrosion by the aggregation of the gold residue on the surface after selective dissolution of silver. Figure 2 demonstrates how the islands grow and the channels between them shrink as the corrosion proceeds, and Fig. 3 shows how annealing at only 720 K after an initial exposure of the alloy to nitric acid causes the islands to grow and merge together to form a connected gold-rich surface layer with residual pits where the merger is incomplete. Similar observations on other silver–gold alloys and also copper–gold alloys over a wide range of compositions and exposed to a wide variety of acid environments have confirmed that the island nucleation and growth is common.

These corrosion-induced micromorphological changes can be explained on the basis of a model in which the selective dissolution of silver produces an atomic roughening of the surface

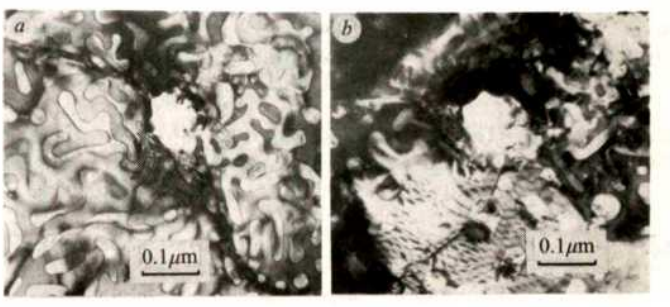


Fig. 3 Transmission electron micrograph of 50:50 Ag–Au alloy: *a*, after corrosion for 60 s in 50% nitric acid and then *b*, after annealing for 60 s at 720 K.

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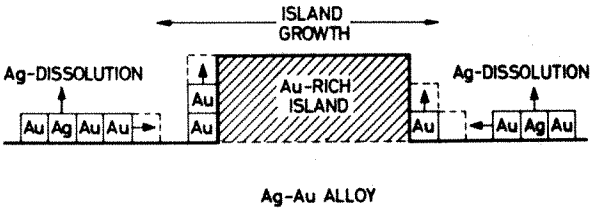


Fig. 4 Gold island growth by the surface disordering, reordering mechanism.

of the alloy (surface disordering). The surface disorder may be regarded as a random distribution of surface silver vacancies or surface gold adatoms, depending on whether the alloy is gold-rich or silver-rich. As the corrosion proceeds the surface reorders by island nucleation and growth from the high concentration of gold adatoms. This process is illustrated in Fig. 4. Finally, the islands grow into an interconnected structure resulting in a very thin epitaxed surface coating of gold.

Although these observations are made on single crystal, high purity alloys, specially prepared for corrosion studies[5], the surface disordering, reordering model can readily be extended to the more practical situation of depletion gilding of castings. The micromorphology of the gold-rich surface layer will vary in detail according to alloy composition, type and strength of acid used, local changes of composition and alloy microstructure at grain boundaries[1], but the final result is expected to be a thin tenacious coating of gold with imperfections which can be subsequently removed by a burnishing treatment.

It would be interesting to carry out a detailed micromorphological study of this kind on binary and ternary silver, copper and gold alloys after corrosion in reactive environments similar to those thought to be used by the ancient Indian goldsmiths.

I thank Professor Colin Goodman for drawing my attention to the Royal Academy Exhibition *The Gold of El Dorado* which caused me to connect my work on the corrosion morphology of silver–gold alloys with the art of depletion gilding.

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Incorporation of elemental sulphur in coal as organic sulphur

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Organic sulphur can represent over 50% of the total sulphur content in coal[1], but little is known about the nature of this organic sulphur because of the chemical complexity of the coal structure and heterogeneity of the maceral assemblages forming the coal matrix. This report focuses on the mode of incorporation of organic sulphur in the very early stages of the coal-forming process. Some organic sulphur in coal originates from the sulphur-containing amino acids that are present in source plants in the peat-forming stage, but we demonstrate here that some of the organic sulphur in coal can actually originate from an inorganic sulphur source, such as elemental sulphur.

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The coal-forming process is a geochemical continuum that includes: peat → lignite → bituminous coal → anthracite coal. Contemporary coal progenitors can be found in peat-forming systems of the Okefenokee Swamp, Georgia and the Everglades Swamp, Florida[2]. Each peat-forming system is a dynamic community composed of plants, surface litter, water, minerals, microorganisms and peat; in the case of the Okefenokee, its present vegetation is similar to that which gave rise to the huge Braunköhle accumulations in Germany and some of the lignites in the Dakotas[3,4].

Virtually all the sulphur in some coals can be accounted for in the peat-forming stage of coal formation[5]. The forms of sulphur that have been found in peat include pyrite, sulphate, hydrogen sulphide, elemental sulphur, carbon bonded (C–S) sulphur, and ester sulphate (C–O–S)[5,6]. Organic sulphur is the major variety of sulphur in virtually all peats studied: it represents over 60% of the total sulphur. One origin for the organic sulphur in peat is the sulphur-containing amino acids such as methionine, cystine and cysteine that are contributed to the sediment by decaying plants and associated microflora and microfauna, but less than 40% of the total organic sulphur can be accounted for as sulphur associated with amino acids[7]. As a function of depth, fewer sulphur-containing amino acids were observed in peat from a marine environment, even though total organic sulphur increased substantially with depth.

Initially, it was thought that the sulphur-containing amino acids were being incorporated via chemical reaction into the various organic fractions of the peat, such as humic and fulvic acids. Yet, in many peat profiles, organic sulphur increased to levels that were substantially greater than the source plant materials and certainly considerably higher than that found in the upper levels of the peat profile, suggesting other sources of organic sulphur in the peat. Substantial amounts of elemental sulphur are observed in some peat-forming systems[5], but virtually none in coal[1]. The elemental sulphur is probably of microbial origin[8].

To test the idea that elemental sulphur serves as a source of organic sulphur in peat, and hence coal, low sulphur peat (0.10%) from the Okenfenokee Swamp was fractionated to produce humic acids. Humic acids were chosen for the reaction since they are recognised progenitors of some of the more important macerals in coal and the organic sulphur of coal is associated with the maceral assemblage. A 1-g aliquot of the prepared humic acid was placed in a round bottom flask and 100 ml of chloroform and 5 μCi of ^{35}S were added to each flask. The contents of the flasks were refluxed for 6 to 48 h, then cooled, filtered and rinsed thoroughly with chloroform and then 1M HCl (chloroform removed any unreacted elemental sulphur, and the HCl dissolved any FeS that may have formed). The total ^{35}sulphur and inorganic ^{35}sulphur were determined using the Johnson–Nishita procedure[9] as modified by us[5,6], and organic ^{35}sulphur by difference. For total sulphur analysis, all the sample sulphur was oxidised to sulphate, and then the oxidised sample was placed on a Johnson–Nishita apparatus; the sulphur in the sample was subsequently reduced to H_2S via a HI-reducing solution; the H_2S was then carried via nitrogen into a zinc/sodium acetate solution, where the ^{35}sulphur became bound as $Zn^{35}S$. An aliquot of this solution was mixed with Insta-Gel and counted in a Packard liquid scintillation counter. For the analysis of inorganic sulphur, the oxidation step, in the sample preparation, was omitted; organic sulphur was determined by difference. It should be noted that any adsorbed sulphur would be detected in the analysis used for inorganic sulphur.

When ^{35}S was allowed to react with the prepared humic acid fraction there was a near linear increase in the organic sulphur content of the humic acid, as a function of time (Fig.1). The conditions of the experiment were mild enough and the chemical reactants were appropriate enough to warrant serious consideration of this reaction as a potentially important source for some of the organic sulphur found in coal. The exact chemical mode of incorporation of elemental sulphur into humic acid is

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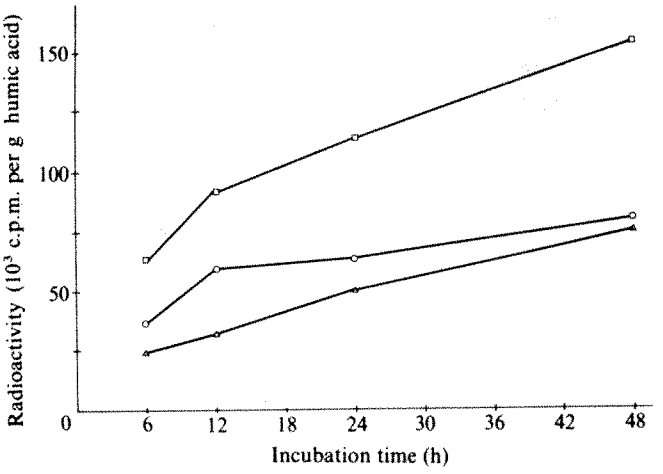


Fig. 1 Uptake of elemental sulphur in the humic acid fraction of Okefenokee peat. □, Total sulphur; ○, inorganic sulphur; △, organic sulphur.

not clear. Sulphur associated with humic acids in marine environments has been shown to be isotopically light, suggesting a microbial origin[8,10]. Elemental sulphur in sediments is thought to originate from the microbial oxidation of H_2S, and H_2S is known to originate from the microbial use of sulphate as a terminal hydrogen acceptor[8,11].

Further evidence of interactions of elemental sulphur with the organic matter in peat comes from the findings that elemental sulphur will react with certain amino acids[12], cholesterol[13] and ethyl benzene[14] to produce organic sulphur compounds. However, in all these cases, reactions were carried out at temperatures at 130 °C or above; in some cases, closed tube conditions with the addition of oxygen and catalysts were used. At these temperatures it is possible that H_2S was formed and hence was the actual reactant chemical as opposed to the original elemental sulphur. Experiments performed in the present study more closely approximate the environmental conditions that elemental sulphur and peat are subjected to in their diagenetic and metamorphic routes to coalification. The exact mechanism for the reaction of elemental sulphur with peat is not known—many carbonium ion and free radical mechanisms are possible, even at the relatively low temperatures of this experiment[15].

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H_2S incorporation in coal precursors: origins of organic sulphur in coal

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Of the forms of sulphur in coal—organic, pyrite, and sulphate—the organic sulphur component is the most difficult to remove before coal can be used. Organic sulphur, which is generally regarded as sulphur in a carbon–sulphur linkage, can represent >50% of the total sulphur found in some coals[1]. We describe here an investigation into the origin of the organic sulphur in the coal-forming sequence which includes: peat → lignite → bituminous coal → anthracite coal. Because of the chemical heterogeneity and associated chemical complexity of working with either bituminous or anthracite coals, the earliest stage of coal formation, that is the peat-forming stage, was studied. Modern progenitors of coal from the Okefenokee Swamp were studied, as these peat-forming systems closely approximate ancient systems that eventually gave rise to huge coal deposits[2–4]. While amino acid sulphur from source plants is an important progenitor of organic sulphur in peat, it was found that H_2S can react with the organic matter in peat to produce organic sulphur—a source of organic sulphur in coal that has not been previously discussed.

The peat-forming stage is a crucial time for sulphur incorporation in the coal-forming process; virtually all of the organic and pyritic sulphur in high and low sulphur coals can be accounted for at this stage[5,6]. Modern peat-forming systems, such as the marine-derived mangrove peats in the Florida Everglades, can contain as much as 10% total sulphur, of which 30–50% is organic sulphur. Sulphur must accumulate in peat as its content is sometimes an order of magnitude higher than that found in associated plants. Sulphur-containing amino acids, such as cysteine, methionine and cystine, found in both higher plants (source materials for the peat) and microorganisms (degraders of the peat), have been viewed as the source of organic sulphur found in coal[6]. Analysis of sulphur-containing amino acids in mangrove-derived peats shows that only 20–40% of the organic sulphur in peat is present as sulphur incorporated in amino acids[7,8]. Thus, the source of a major portion of organic sulphur in coal formation is in the peat-forming stage, yet the origin of this organic sulphur has not been fully explained. In walks through the peat during a field trip, a strong smell of H_2S was common; the source of this H_2S is probably microbial. *Desulfovibrio* occurs in anoxic environments and this bacterium uses sulphate as a terminal hydrogen acceptor, producing H_2S during the process[9]. In a series of experiments in our laboratory it was observed that as much as 4 mg S per kg wet peat per day (as H_2S) was produced from mangrove-derived peats.

These observations, and the fact that the organic sulphur increase in peat is not necessarily related to the associated source plants, caused us to design a series of experiments to evaluate the possibility that some of the organic sulphur found in coal may derive from the interaction of H_2S with the organic matter in peat. Aliquots from a large sample of an homogenised wet peat sample were placed in a series of desiccators; the atmosphere over the peats was evacuated using a water pump. The peat used in this study originated from core material in the Chesser Prairie area of the Okefenokee Swamp; it had a sulphur content of 0.25% on a dry basis. An $H_2^{35}S$ generating system was constructed that allowed an $H_2^{35}S/N_2$ atmosphere to be introduced over each peat sample. In operation the peat was blanketed in the desiccators with a $H_2^{35}S/N_2$ atmosphere as follows:

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Table 1 The distribution of labelled organic sulphur in organic peat fractions

Time (h)	Water soluble	Benzene/methanol	Humin	Humic acid	Fulvic acid
72	5.64	—	81.84	12.03	0.49
168	3.90	0.007	40.84	55.10	0.16
504	1.08	0.14	7.59	90.65	0.44
840	1.00	0.11	25.19	73.63	0.04
Mean	2.90	0.09	38.86	57.85	0.28

(1) $Na_2^{35}S$ was put into a two neck round bottom flask, which had one side adapted for the introduction of H_3PO_4 and the other side adapted for a gas inlet/outlet; (2) the system was purged with N_2 gas; (3) H_3PO_4 was introduced on top of the $Na_2^{35}S$; (4) the entire system was closed to allow for $H_2^{35}S$ build-up; (5) stopcocks were opened that would allow for the passage of nitrogen though the $H_2^{35}S$ generating chamber and conduction of the $H_2^{35}S/N_2$ mixture into the previously evacuated desiccators; and (6) the desiccator atmosphere was filled with N_2 until ambient pressure was reached.

After various intervals (72–840 h) an individual desiccator was opened and the peat was fractionated into five components; water soluble, benzene/methanol soluble, fulvic acid, humic acid and humin. Each fraction was then analysed for both total and inorganic labelled sulphur. Note that the procedure used to analyse inorganic sulphur would also measure any hydrogen ^{35}sulphide that was adsorbed along with any elemental ^{35}sulphur, ^{35}sulphate, ^{35}sulphides, ester ^{35}sulphate, and so on, but does not measure carbon bonded (organic) ^{35}sulphur. Thus organic ^{35}sulphur[10] was calculated as the difference between total ^{35}sulphur and inorganic ^{35}sulphur.

Table 1 shows the percentage of total organic ^{35}sulphur activity in the peat as a function of organic fraction and as a function of time. There are several interesting points: (1) H_2S reacts with peat to produce organic sulphur; (2) H_2S is incorporated into the organic sulphur fraction at room temperature and it occurs in a relatively short period of time—compatible with diagenetic processes occurring in modern organic sediments; (3) the humic acid fraction—an important precursor to coal maceral(s)—shows the largest amount of organic sulphur incorporation, even though it represents <20% of the total organic matter in peat; (4) the humin fraction, which represents 70% of the total organic matter, has an average of only 38.8% of the total organic sulphur associated with it; (5) there is little organic sulphur incorporation into the water soluble fraction—the water soluble fraction represents a pool of 'free constituents' which should be available for reaction; (6) little organic sulphur incorporation is found in the fulvic acid fraction—previous studies have shown that 75% of the sulphur associated with the fulvic acid fraction is in the form of ester-sulphate[8]. A plot of the uptake of $H_2^{35}S$ as organic sulphur in the humic acid fraction, showed monotonic production of organic sulphur in the organic matter as a function of time.

The chemical nature of the bonding involved in the reaction of H_2S with organic matter is difficult to assess. It is possible that H_2S is converted to another species, which is the reactive agent[11–13]. However, there are reports which demonstrate H_2S incorporation in (1) petroleum[14], (2) organics in natural waters[15], (3) non-sulphur-containing amino acids[16] and (4) unsaturated fatty acids[17]. Also, Kaplan and co-workers have indicated that the sulphur associated with marine-derived humic acids is isotopically light sulphur which is microbially-derived[18]. The present study certainly supports this view and points to at least one of the microbially-derived sources for some of the organic sulphur in coal. The study also shows that important premaceral materials, such as humic acids, obtain an organic sulphur component at a very early point in their diagenetic history from a source that is not originally carbon-bonded. Finally, the importance of this type of reaction in the overall biogeochemical cycle of sulphur needs further investigation.

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Are solar spectral variations a drive for climatic change?

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Observations of the Sun from satellite and rocket platforms have provided good evidence that solar radiation varies noticeably in the UV portion of the spectrum over a solar rotation period of 27 d[1,2]. There is also less conclusive evidence for such variations on the time scale of the 11-yr sunspot cycle[1,2]. We investigate here the possible effects of these UV variations on atmospheric ozone content and climate for time scales encompassing the 27-d solar rotation period, the sunspot period, twice this period (solar magnetic period) and much longer times. We conclude that (1) solar UV variations on time scales of weeks to months can occasionally perturb total ozone and stratospheric temperatures by noticeable amounts, but they result in only very minor changes in the troposphere; (2) the above cited estimates of UV variation over the 11-yr solar cycle are inconsistent with measured changes in total ozone; (3) with UV variations being limited by long-term trends in total ozone, only very small variations in surface temperature are expected over the 11-yr solar cycle, but possibly significant ones could occur over the 22-yr cycle; (4) solar spectral changes might be partially responsible for century-long variations in climate, which have been empirically correlated with changes in solar activity[3,4].

To distinguish clearly the solar spectral change mechanism for climate modification from ones involving luminosity changes, we alter the visible spectrum slightly ($\lesssim$ few tenths %) so that the integrated solar radiant output is an invariant. We note that very severe upper bounds (0.03%) have been placed on the variation of the solar constant on time scales of months[5], despite the occurrence of measurable UV variations on this same time scale[1,2]. Somewhat larger upper limits have been set on the change in solar luminosity between 1969 and 1976 (0.15%) (ref. 6) and from 1923 to 1952 (0.17%) (ref. 7).

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Our studies of the relationship between solar UV variations and atmospheric ozone content and atmospheric temperatures differ from others[8-10] in that we estimate the impact of such variations on tropospheric temperatures, we hold the total luminosity constant, and we examine the dependence of the ozone variations on the forcing period.

The calculations were performed with a three-step iterative procedure for UV variations having a period long compared with the time required for the ozone profile to reach a steady-state solution (~1 yr). First, a one-dimensional time-marching photochemical model with vertical transport and a fixed temperature profile was used to determine the temporal variation of the ozone and nitrogen dioxide concentrations from the ground to 120 km altitude[11]. Next, the results of the photochemical calculations were used in a numerically accurate, vertically inhomogeneous, multiple scattering computer program to determine the globally averaged, solar energy deposition profile[12]. Finally, this profile was incorporated into a one-dimensional radiative–convective equilibrium program to evaluate the globally averaged temperature structure[13]. Because of the temperature dependence of the photochemical rate coefficients, we repeated the three-step calculations several times, with the temperature estimates obtained from the third step used as starting conditions.

A different procedure was followed for UV variations having a period less than the ozone relaxation time scale. In such cases the driving period is much smaller than the thermal inertia time constant for the land–ocean–troposphere system (~5 yr (ref. 14)). Thus, little change in tropospheric or surface temperatures will occur. For these cases, we used the same one-dimensional time-marching photochemical model, but with a parameterised radiative cooling and heating capability that provided estimates of temperature perturbations in the stratosphere and mesosphere[15].

As we neglect a large number of feedback processes that could alter the final answer[16], our results should be viewed simply as an exploratory study of the sensitivity of the Earth's climate to solar spectral variations. For example, we do not attempt to vary the properties of tropospheric water clouds in response to the UV ozone and temperature changes nor do we allow for dynamical effects.

There are two mechanisms that might cause solar UV variations, each being possibly pertinent for different time periods. One manifestation of solar activity is a region called a plage that covers a small fraction of the surface and is hotter than the rest of the Sun at the same altitude[17]. Because the spectral irradiance in the UV is on the Wien tail of the black-body function, much larger variations can occur in the UV than in the visible due to changes in the area covered by plages. We model this situation—essentially the sum of two black-body functions—by setting the fractional change in the UV spectral irradiance equal to $C \exp(-b(1/\lambda_0 - 1/\lambda)) \sin(2\pi t/P)$, in accord with available broad band observations of solar UV variations over a solar rotation period[1,2] where C and b are constants; λ and λ_0 are wavelength and a reference wavelength (0.175 μm), respectively; and t and P are the time and period, respectively.

The increase of temperature with altitude above the solar photosphere is thought to be due to the upward propagation of energy (such as acoustical waves) from the solar convection zone that is located right below the photosphere[18]. Conceivably, on long time scales (> yr), solar activity variations may be accompanied within the convection zone by variations in the division of the net solar flux between radiative and turbulent net fluxes. If so, alterations in the vertical solar temperature structure, particularly near the solar temperature minimum and at higher altitudes, may occur, without there necessarily being a change in the solar luminosity. Both because UV irradiance is produced near the temperature minimum and because it is situated on the Wien tail of the black-body function, such alterations of temperature structure would preferentially affect the UV output, with the magnitude of the spectral variation depending on the altitude from which the irradiance originates. We model this situation by setting the fractional variation of UV irradiance equal to $C' \exp(-b'(T_\lambda - T_{\lambda_0})) \sin(2\pi t/P)$, in accord with limited observations over the 11-yr solar cycle[1,2] where C' and b' are constants and T_λ and T_{λ_0} (4,750 K) are the brightness temperatures of the Sun at wavelengths λ and λ_0, respectively.

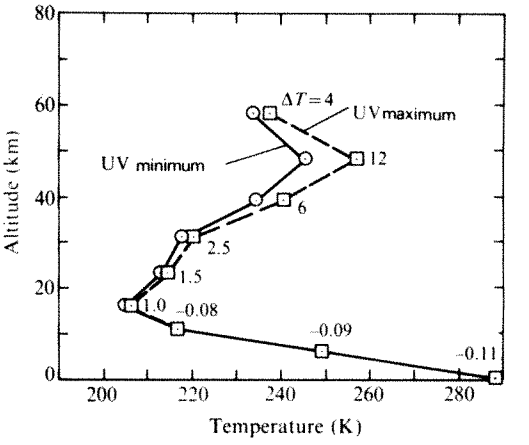


Fig. 1 Steady-state temperature profiles for times of UV maximum and minimum for long period perturbations (≫10 yr). Also shown are the temperature differences, ΔT, between these two cases.

The above alterations of the monochromatic solar fluxes were done only at wavelengths shorter than 0.29 μm, while the fluxes at longer wavelengths were altered uniformly in the opposite direction to hold the total luminosity constant. A value of 0.29 μm for the transition wavelength was selected as UV variations shortward of 0.29 μm have been reported[1,2], while severe limits have been placed on variations at visible wavelengths[7].

We have carried out a series of calculations with the brightness temperature parameterisation of the spectral variations and with C' and b' chosen to generate UV variations consistent with the reported 11-yr variations[2] ($C' = 0.4$; $b' = 0.02$). For UV variations having periods >1 yr, calculated peak-to-peak changes in ozone column density closely approach an asymptotic limit. Periods of 27 days and 6 months are characterised by peak-to-peak changes that equal about one-third and two-thirds of this asymptotic limit, respectively. Vertical transport limits the response of the system over these short time scales. We obtained very similar variations in ozone amount when the wavelength parameterisation was used and when the ratio of the amplitudes of the UV variation at 0.29 μm and 0.175 μm was varied by varying b', but the amplitude at 0.175 μm (C') was held fixed.

The solar energy deposition profile is influenced by changes both in the trace gas constituents and in the solar spectrum. For all the driving periods considered, UV minimum is characterised by a smaller amount of energy absorbed in the stratosphere, a higher global albedo, and a larger energy deposition below the tropopause. Decreases in the ozone column density and the UV flux are responsible for these trends. Some of the sunlight not absorbed in the stratosphere during UV minimum is Rayleigh scattered back to space—hence the higher albedo—while the remainder is transmitted to the troposphere—hence higher deposition below the tropopause.

Figure 1 shows the radiative–convective equilibrium temperature profiles at times of UV minimum and maximum flux for the longest period cycles (≫10 yr). As might have been anticipated from the above discussion of solar energy deposition, the stratospheric temperatures increase from UV minimum to maximum, with the largest changes occurring near the stratopause. However, the tropospheric temperatures decrease, with the surface being about 0.1 K cooler during UV maximum.

Table 1 Predicted changes in atmospheric parameters due to solar spectral variations¶

Period	Epoch	Constraint* (refs)	ΔUV*	ΔO_3^*	δT^*_{strat} (K)	δT^*_{surf} (K)	ΔS^*_{trop}
27 day	1969†	ΔUV (1)	0.05	0.003	0.1	−0.00003	−0.0002
6 month	1970†	ΔO_3 (19, 20)	0.15	0.013	2	−0.0006	−0.0005
11 yr	1966‡–70†	ΔUV (2)	1.5	0.13	12	−0.06	−0.005
11 yr	1970†–75‡	ΔUV (2)	1.5	0.13	12	−0.06	−0.005
11 yr	1966‡–70†	$\Delta O_3^{\|}$ (19)	0.4	0.035	3	−0.02	−0.001
11 yr	1970†–75‡	$\Delta O_3^{\|}$ (19)	0.4	0.03	3	−0.02	−0.001
22 yr	1958†–69†	$\Delta O_3^{\|}$ (19)	0.8	0.07	6	−0.06	−0.003
Century	Late 17th§ late 18th centuries	$\delta T^{\|}_{surf}$ (34)	3 #	0.26	23	−0.2	−0.01
Threshold values*		(refs 19–22)		±0.003	±0.5	±0.05	±0.01

* See text for definition.
† Close to sunspot maximum.
‡ Close to sunspot minimum.
§ Time of the Maunder sunspot minimum.
‖ These values are upper bounds.
¶ In all cases, the ratio of the fractional change in irradiance at 0.175 μm to that at 0.29 μm has been set equal to 5, in accord with refs 1, 2.
We require a larger UV flux at the time of the Maunder sunspot minimum.

Evidently, the increase in downward directed thermal radiation from the stratosphere between UV minimum and maximum, due to the increase in ozone and the higher stratospheric temperatures, is less important for the tropospheric heat balance than the decrease in solar energy deposition below the tropopause. For the 11-yr period, the temperature changes in the troposphere will be only about half of their values for the longer period cases because the response time of the ocean–land–atmosphere system is comparable to the half period of the 11-yr solar cycle[14]. Correspondingly smaller responses characterise the shorter period cases. The computed changes in temperature structure are insensitive to the functional form chosen for the spectral variations, but they are sensitive to both the amplitude of the UV variation at 0.175 μm and the ratio of the amplitudes at 0.29 and 0.175 μm.

To assess the significance of changes in climate induced by solar spectral variations, we introduce the following parameters: δT_{strat}, δT_{surf}, which are the difference in stratopause (~48 km) temperature and surface temperature, respectively, between times of UV maximum and minimum, while ΔS_{trop}, ΔO_3 and ΔUV are the corresponding differences in solar energy deposited below the tropopause, ozone column density and solar UV intensity at 0.175 μm, respectively, divided by the values of these quantities at UV minimum. Table 1 summarises our estimates of these parameters for periods of spectral variation ranging from the 27-day solar rotation period to a century. In each case, an observed value of ΔUV, ΔO_3, or δT_{surf}, as indicated by the 'constraint' column, has been used in conjunction with our model calculations to estimate the values of the remaining four parameters. Table 1 also shows our crude estimates of threshold values for the various parameters, below which a predicted variation would be small compared with known changes in its value. The threshold value is set at a quarter of the known change. The peak-to-peak variations associated with the quasi-biennial oscillation were used to derive the threshold values for δT_{strat} and ΔO_3 (refs 19, 20). Threshold values of ΔS_{trop} and δT_{surf} were obtained from a tidal theory for planetary waves[21], and the globally averaged change in surface temperature over the past several decades[22], respectively.

For changes occurring on time scales of weeks, good correlations have been obtained between solar activity indices and tropospheric circulation parameters[23]. However, the reality of this solar–terrestrial relationship has been challenged because the above correlation is sensitive to which months are included in the analysis[24]; no significant correlation is obtained for some epochs[24,25]; and power spectral analyses of key meteorological variables fail to show a significant peak near 27 days[26]. Since solar UV variations may represent the most energetically important short-term modulation of solar output, it is of interest to estimate the impact of these variations on weather phenomena. As Table 1 shows measured values of ΔUV associated with the 27-day solar rotation are not expected to have caused significant alterations to any of the key atmospheric parameters, with the possible exception of a marginally significant alteration of the ozone column density.

For changes occurring on a somewhat longer time scale, a statistically significant correlation has been found between monthly averaged global ozone amount and solar activity indices[27,28]. The maximum value of ΔO_3 was found to be 0.013, with a characteristic period of about 6 months. According to Table 1, about a threefold increase in ΔUV over the 27-day value is required to achieve this ΔO_3. Even in this case, no significant alteration of the tropospheric parameters δT_{surf} and ΔS_{trop} results. However, small, but significant, changes occur in the stratospheric parameters δT_{strat} and ΔO_3, which could conceivably induce marginal effects in the troposphere through stratospheric–tropospheric coupling processes[29]. We conclude that, at best, UV spectral variations having time scales of weeks to months may occasionally perturb tropospheric weather in a barely noticeable manner.

From comparisons of measurements of solar UV irradiance made by similar broad band photometers carried on rockets and satellites, it has been suggested that the UV irradiance increased significantly from 1966 to 1970, with ΔUV ≅ 1.5 (ref. 2): 1966 was close to sunspot minimum and 1970 was close to sunspot maximum. Similar comparison of data obtained by satellite-borne spectrometers imply a decrease in UV irradiance between 1970 and 1975 of about the same magnitude[2]. Some support for the validity of these estimates of ΔUV for the 11-yr sunspot cycle is given by an apparent agreement between observed ozone and temperature variations in the middle stratosphere during the late 1960s and early 1970s and predicted changes based on these ΔUV estimates[30]. However, there are large error bars associated with this climate data set and it spans only about one sunspot cycle.

Consequently, we have examined additional data to assess further the accuracy of the above values of ΔUV for the 11-yr sunspot cycle. Estimates of the change in average ozone column density[19] for the Northern Hemisphere that cover the actual times of the solar irradiance measurements imply ΔUV ≅ 0.4 and not 1.5 (Table 1). This disagreement is much larger than would be expected on the basis of the error bars assigned to the average ozone column densities. A slightly larger discrepancy results when the less well determined globally average ozone densities are used. Furthermore, the failure of the average ozone

column densities for the Northern Hemisphere and the world to increase from sunspot minimum in 1964 to sunspot maximum in 1958 raises some doubts as to whether the later variance (1964–75) is dominated by an 11-yr period. Also, much smaller changes in middle and upper stratospheric temperatures occurred over the time period of the first set of solar UV measurements (1966–70) than expected on the basis of the claimed UV variation and much smaller temperature changes occurred in the upper stratosphere over the interval of the second set[20,31]. The ΔUV values given by Heath and Thekaekara[2] for the 11-yr solar cycle are, therefore, probably large overestimates of the true variation. According to Table 1, if all the variance in the average ozone column density between 1966 and 1970 and between 1970 and 1975 is ascribed to 11-yr UV variability, then significant changes in δT_{strat} and ΔO_3 result, but insignificant changes in δT_{surf} occur.

There seems to be a 22-yr periodicity in certain climate phenomena, such as the recurring drought on the high plains of the US[32] and in other climate indicators[33]. The time variation of average ozone densities between 1958 and 1976 may be dominated by a 22-yr period rather than an 11-yr period[19]. If all the variance in ozone amount between 1958 and 1969 is ascribed to 22-yr solar UV variation, then large changes in δT_{strat} result as well as a possibly significant variation in δT_{surf}. Conceivably, this value of δT_{surf} is large enough to account for the above cited 22-year climate changes.

Strong correlations have been shown to exist between century-long times of anomalous solar activity and climate anomalies, with periods of low solar activity corresponding to colder epochs[3,4]. According to Table 1, if all the observed δT_{surf} for a period encompassing the Maunder sunspot minimum is ascribed to solar spectral variations, very sizeable changes in all the key variables result. It is reasonable to ascribe at least some fraction of δT_{surf} to this mechanism in view of the observed changes in the structure of the uppermost regions of the Sun associated with the Maunder minimum[3,4]. Finally, aside from the significant temperature perturbations perhaps associated with longer term solar spectral variability, biologically significant changes in ozone amount also seem to be implied.

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Neogene explosive volcanicity, temperature and glaciation

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Recent theoretical[1–6] and observational[7–10] demonstrations of depression in surface temperature of the Earth due to explosive volcanic eruptions have provided a more exact basis for speculations that explosive volcanicity may have had a climatic impact[11–15] during periods over the past 2 Myr. In this letter, broad volcanic–climate relationships are assessed over a longer period by comparing the dates of global explosive phases and of volcanic–plutonic phases with paleotemperature and glaciation records from the Neogene. Analysis of these records indicates significant relationships between the explosive and volcanic–plutonic phases and Neogene temperature decline and also a correspondence between the three greatest explosive and volcanic–plutonic phases and glacial expansion. The second highest global explosive peak was coincident with recently suggested dates of around 3.2 Myr for the initiation of the late Cenozoic glacial age. Further testing of these relationships is necessary to resolve inconsistencies in the late-middle to early-late Miocene and in the late Pliocene.

To determine patterns of temperature trend for the Neogene, I examined six palaeotemperature curves[16–21] for trend during geological epochs and their subdivisions and for trend on the absolute time scale of Berggren[22]. This examination indicated that the six studies were in agreement in showing two major periods of temperature decline, the middle Miocene and the late Pliocene or early Pleistocene with two studies[16,17] also showing substantial declines, and another study[18] a minor decline in the late Miocene. There was also predominant agreement on a temperature increase during the early Miocene and the middle Pliocene. Temperature trends, using Berggren's scale[22], from Figs 5 and 6 of Kennett[16], Fig. 16 of Margolis *et al.*[17], Fig. 2 of Buchardt[18] and Fig. 4 of Savin *et al.*[19] are shown in Table 1. Results from the other two palaeotemperature studies[20,21] were not included because they were less sensitive and were repetitive of the four presented curves. Table 1 also includes estimates of the occurrence of major glacial expansion periods which included dates of 16–14 Myr (ref. 16), 5–6 Myr (refs 16, 23) and 4.7–4.3 Myr (ref. 23) for Antarctica and 16 Myr (ref. 24) for the Northern Hemisphere.

The palaeotemperature and glaciation data in Table 1 were compared with two independent estimates of Neogene volcanic patterning. The first, by Kennett *et al.*[25], was based on a Pacific Basin marine ash survey which found four major Neogene explosive episodes that occurred (in descending magnitude) in the Quaternary (2–0 Myr), the middle Miocene (16–14 Myr), the latest Miocene to early Pliocene (6–3 Myr) and the late Miocene (11–8 yr). These patterns are similar to those of a global marine ash survey conducted by the same authors[25] and summarised in their Fig. 6. The second estimate of patterning of Neogene volcanicity was based on a global literature review which I made of 1,084 exactly dated volcanic and plutonic events, both terrestrial and marine. Examination of the distribution of these events suggested that there were two major and one minor Neogene volcanic–plutonic phases (number of volcanic–plutonic events per 10^5 yr in parentheses) 3.3–0 Myr (17.7), 16.0–13.0 Myr (4.4) and 6.4–5.0 Myr (4.1) and three intervening periods of lesser volcanic–plutonic activity, 19.9–16.1 Myr (2.1), 12.9–6.5 Myr (2.7) and 4.9–3.4 Myr (3.6). Volcanic–plutonic activity peaks occurred at 19.5–19.4, 16.6–16.5,15.7–15.6, 15.2–15.0, 14.6–14.5, 13.5–13.4, 11.4–11.3, 10.0–9.8, 8.3–8.2, 6.3–6.1, 5.8–5.7, 5.1–5.0, 3.1–3.0, 2.7–2.6, 2.4–2.3, 1.9–1.8, 1.2–1.0, 0.6–0.5 and 0.3–0.1 Myr.

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Table 1 Neogene volcanic activity, glacial expansion and global palaeotemperature trends

Myr BP	Volcanic activity: Explosive	Volcanic activity: Volcanic–plutonic	Glacial expansion	Palaeotemperature: *a*	*b*	*c*	*d*
20–19				−	+	+	+
19–18				−	−	+	+
18–17				+	−	+	+
17–16				+	−	+	+
16–15	×	×	×	−	−	+	−
15–14	×	×	×	−	−	−	−
14–13		×		−	−	−	−
13–12				−	−	−	−
12–11				+	−	−	−
11–10	×			−	−	−	−
10–9	×			−	−	−	+
9–8	×			−	−	−	+
8–7				+	−	+	+
7–6				−	−	+	+
6–5	×	×	×	−	−	−	+
5–4	×		×	−	−	−	+
4–3	×			−	+	+	+
3–2		×		+	−	−	−
2–1	×	×	×	+	−	−	−
1–0	×	×	×	−	−	−	−

Explosive episodes from Kennett *et al.*[25]; volcanic–plutonic episodes from global literature survey. Glacial expansion periods as in text. Palaeotemperature trends: *a*, Kennett[16]; *b*, Margolis *et al.*[17]; *c*, Buchardt[18]; *d*, Savin *et al.*[19]. Plus indicates an increasing, and minus a decreasing global temperature.

The geological occurrence of the four explosive phases in the Quaternary, middle Miocene, latest Miocene to early Pliocene and the late Miocene, as described by Kennett *et al.*[25], overlapped the three main global temperature decline periods outlined above which occurred in the late Pliocene to early Pleistocene, middle Miocene and the late Miocene. During the two greatest explosive episodes, from 16–14 and 2–0 Myr, 14 of 16 temperature estimates showed a decline. For the moderate explosive episodes of 11–8 and 6–3 Myr, there were 17 of 24 temperature estimates that declined. In the remaining, non-explosive intervals, 21 of 40 temperature estimates showed a decline. To test the validity of these patterns, the temperature data in Table 1 were compared with the four Kennett *et al.* explosive episodes, using a fourfold contingency table. The result gave a χ^2 of 5.5 ($P < 0.02$) which suggests a connection between the occurrence of Neogene explosive episodes and a tendency towards temperature decline. A fourfold contingency χ^2 test was also applied to the volcanic phases which occurred at 16–13, 6–5 and 3–0 Myr in the global volcanic–plutonic survey and the temperature data in Table 1. The result gave a χ^2 of 8.1 ($P < 0.005$), again suggesting a Neogene volcanism–temperature relationship.

Examination of the possible volcanism–temperature relationship in Table 1 indicates an anomaly in the late Middle to early late Miocene around 14–11 Myr during which time 11 out of 12 palaeotemperature data showed a decline during a period of low explosive activity. Data in the global survey of Kennett *et al.*[25] indicate, however, that the level of ash produced in this period was only moderately less than in the preceding and succeeding explosive episodes which suggests that the trend of temperature decline which began during the early middle Miocene may have been sustained by the moderate activity from 14 to 11 Myr.

There was a correspondence in Table 1 between the major Neogene glacial expansion periods at 16–14, 6–4 and 2–0 Myr and both the three highest Neogene explosive episodes and the three volcanic–plutonic phases. The apparent lack of a major glacial expansion at the explosive episode around 11–8 Myr could relate to the explosive level during this interval which had the lowest activity of the four Neogene episodes designated by Kennett *et al.*[25].

The second highest Neogene explosive peak in Kennett *et al.*[25] (Fig. 2) was from 3.5 to 3.0 Myr. Kennett *et al.*[25] in noting this secondary explosive peak stated that there were also many lavas of this age in the circum-Pacific, but that there were not enough data to determine whether this 3.5–3.0 Myr peak was distinct from, or part of, the explosive episode from 6 to 3 Myr. The peak around 3.5–3.0 Myr is also present in a global survey of volcanic–plutonic activity which I have made and in local explosive studies including a well-dated upswing which began between 3.2 and 3.1 Myr in Japan[26]. This major explosive peak from around 3.5–3.0 Myr was concurrent with an extensive and globally well-distributed array of estimates of major temperature decline around 3.2–3.1 Myr (refs 26–30) and glacial expansion around 3.5–3.1 Myr (refs 31–35) which are currently regarded[28] as the date for the initiation of the late Cenozoic glacial age.

From around 3.0 Myr to the present, the global explosive data[25] show a period of low output from 3.0 to 2.0 Myr and then a maximum activity from 2.0 to 0 Myr. As the climatic data suggest that there was a period of cooler temperatures and cyclic glacial expansions from around 3.2 to the present[28], the low global volcanic record from 3.0 to 2.0 Myr may contradict the volcanic climatic hypothesis. Regional studies[36–38] from ocean cores in the North Pacific indicate, however, that there was a generally high level of explosive activity during the 3.0–2.0 Myr interval with a major pulse around 2.5–2.3 Myr. Further and more sensitive dating of the global explosive data may resolve this inconsistency.

The Neogene volcanism–climate relationships shown above were assessed under the assumption that it is the total level of explosive activity that may have had a climatic impact. It is conceivable, however, that a relatively few single or closely spaced massive eruptions could have acted as trigger devices[39] for the 'instantaneous glaciation' mechanism and that it is the occurrence of these massive eruptions which is climatically significant. This possibility is not contradicted by the demonstrated correspondence between the Neogene explosive episodes, when massive eruptions more likely occurred, and the climatic parameters in Table 1.

Since writing the above, I received a manuscript from Kennett[40] which has conclusions broadly similar to those presented here, and includes the statement that the four explosive phases outlined in Kennett *et al.*[25] coincide with "critical developments in the climatic and glacial evolution of the earth".

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India's and Australia's pole path since the late Mesozoic and the India–Asia collision

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The Indian apparent polar wandering path (APW) for the late Mesozoic–Cenozoic period has been too poorly defined to use as a constraint to establish the timing of collision of India and Asia. Here we update the Indian APW on the basis of new palaeomagnetic data from DSDP cores and from Baluchistan (Pakistan). This new synthesis details the late Mesozoic and Cenozoic northward motion of India, and supports geological evidence for an initial collision with Asia during the late Palaeocene–early Eocene.

Recent attempts to trace India's northward motion and collision with Asia have used two approaches. Seafloor spreading data from the Atlantic and Indian Oceans were used to detail the relative Indian–Eurasian motion[1]. According to this rather generalised approach intimate contact between the two continental masses was established by Eocene–Oligocene time (~40 Myr), based on a reduction in the rate of relative motion from an initial 10 to 16 cm yr^{-1} to about 5 cm yr^{-1}.

On the other hand, using the relative motion data of the Indian, Australian and Antarctic plates[2–4] to reconstruct the Indian–Australian relative motion and the Australian APW[5–7] for positioning the Indian plate relative to the pole, others have argued[8,10] that initial collision occurred during late Palaeocene–early Eocene time (~55 Myr; ref. 9). India's northward motion reduced at that time from 15–20 cm yr^{-1} to <4–6 cm yr^{-1}, which supports earlier advanced geological arguments[10] for a pre-Middle Eocene timing of collision.

The DSDP pole positions[11] were obtained as 10 Myr averages of individual pole positions, determined from (1) mean palaeolatitudes at a given site and at a given age corrected for a systematic downwards deflection due to absence of declination control[12,13] and from (2) declinations at a given site and at a given age simulated[11] from seafloor spreading data as summarised by Powell *et al.*[14] The Baluchistan data in part come from an allochthonous structural unit, but results have been corrected for a well determinable clockwise rotation[15].

This new Indian APW (Fig. 1) facilitates direct tracing of India's northward movement (Fig. 2*a*). New palaeomagnetic data from Ladakh (north-west Himalaya[16]), north of the Indus–Tsangpo suture zone, have established the palaeoposition of the Ladakh segment of the Kohistan–Ladakh island arc (Fig. 2*b*, refs 17, 18) before collision and during cooling (K–Ar ages 45–49 Myr, see ref. 16) after its collision with northward-moving continental Indo–Pakistan. Comparison of Fig. 2*a* with 2*b* evidently supports the postulated pre-Middle Eocene data of collision[8,10]. On the basis of geological evidence from north-western Indo–Pakistan this postulation can now be refined as initial collision between continental Indo-Pakistan and an island arc off southern Asia, which occurred most probably during the late Palaeocene–early Eocene[9,11,16,19,20].

The former southern boundary of Asia, juxtaposed to India, is not well defined, but can be taken as the eastwards continuation of the Zagros–Makran convergence zone[9]. The 2–10 °N palaeolatitude of the Ladakh segment of the Kohistan–Ladakh island arc before and on initial collision, is far to the south of the contemporaneous position of this extrapolated Zagros–Makran convergence zone relocated according to Eurasian palaeomagnetic data[21]. Closure of a hypothesised marginal basin along the further northwards positioned Shyok zone[17] can possibly be related to a reduction in the rate of the Indian–Eurasian relative movement at the Eocene–Oligocene time boundary as interpreted elsewhere[1,12].

Relocation of India according to Australian palaeomagnetic data and plate relative movement, disagrees (but just not significantly at the 95% level) for at least the early Tertiary period (Fig. 2*c*) with direct palaeolatitudinal observations from the Indian plate (Fig. 2*a*) and from Ladakh (Fig. 2*b*). India's indirectly obtained palaeoposition according to the Australian Barrington Volcano result (Fig. 2*c*; 52 Myr) is about 1,000 km further north than its directly observed position at the time of initial collision. The DSDP core data from the Indian plate and the results from Ladakh are in mutual agreement for the early Tertiary. Therefore, the above discrepancy has to be explained either by an error in the relative-motion data or by an inaccuracy in the Australian APW. The scale of the discrepancy seems beyond that which it is reasonable to expect in the relative motion data. One of us (J.W.P.)[12] previously indicated that an age of the Barrington Volcano about 10 Myr younger than the determined 51.7±0.7 Myr (ref. 5) would resolve the discrepancy. K–Ar ages as young as 43 Myr were reported for this volcano, but were not considered to have significant bearing on the age of the palaeomagnetically studied flows (ref. 5 and I. McDougall, personal communication). Therefore, the dis-

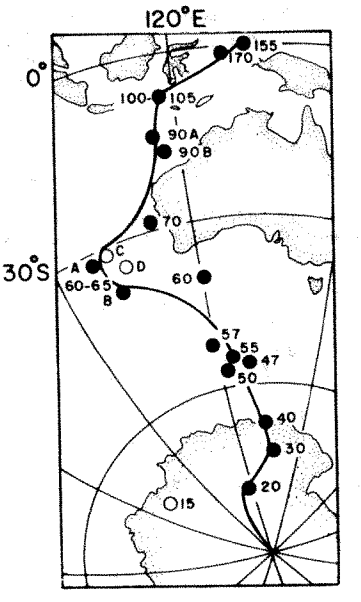


Fig. 1 APW for India based on data from continental Indo-Pakistan and DSDP core data from the Indian plate (Table 1). The ages indicated represent radiometric ages or the mean of stratigraphic age ranges as used in the determination of transferred pole positions (Table 1, Fig. 3*a*). ○ The Murree–Siwalik pole position[22] from the allochthonous Salt Range–Potwar Plateau structural unit, and the pole positions from the Lower and Upper Deccan Trap successions (60–65 C and D respectively) determined from maximum grouping of a density distribution of individual poles[11] as alternatives to Fisherian mean pole positions (60–65 A and B, respectively). Linear polar projection.

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Table 1 Late Mesozoic and Cenozoic palaeomagnetic south pole positions for India and Australia

Formation	Age* (Myr)		Original†			Transferred		Ref.
			Lat (°S)	Long (°E)	$\alpha 95$ (°)‡	Lat (°S)	Long (°E)	
A1) Australia, also transferred to India								
Cygnet alkaline complex	98	(98)	53	156	10	14	124	23
Mt Dromedary complex	93	(93)A	56	138	9	12	114	24
Mt Dromedary complex	93	(93)B	56	153	10	16	121	25
Bunbury basalt	>90–100	(90)	49	161	10	14	131	25
Morney profile	Maastrichtian–Lower Eocene	(57)	60	118	4	43	109	26
Older volcanics Victoria	Paleocene–Eocene?	(55)	61	144	17	53	133	27
Barrington volcano	51.7	(51.7)	70	126	5	65	123	5
Liverpool volcano	33.7	(33.7)	71	96	5	70	98	5
Canaway profile	Upper Oligocene?	(28)	74	115	7	73	117	26
Lavas and Dykes, SE Queensland	20–22	(22)	78	86	12	78	90	24
Tweed volcano	20–22	(21)	72	121	5	71	121	28
Main range volcanics	20–22	(21)	77	98	7	76	100	28
Nandewar volcano	17–18	(17.5)	78	84	5	78	87	5
Newer volcanics Victoria	0–5	(2.5)	87	86	2	87	87	29
A2) Mean Cenozoic results for Australia, also transferred to India								
	95	(95)	55	147	7	13	119	30
	40–60	(50)	69	131	5	64	127	6,7
	35–25	(30)	69	92	4	68	94	6,7
	25–20	(22.5)	77	111	4	77	113	6,7
	20–15	(17.5)	79	66	4	79	69	6,7
B1) India, also transferred to Australia								
Loralai limestone§	middle Jurassic	(170)	2	128	8	34	183	15
Chiltan limestone	Oxfordian to Lower Kimmeridgian	(155)	1	132	10	31	184	15
Rajmahal traps	100–105	(102.5)	8	117	4	34	151	11
Goru Fm-Parh Lst Sanjawi§	Aptian–Albian to Santonian–Campanian	(90)	18	117	3	57	160	15
Goru Fm-Parh Lst Quetta	Aptian–Albian to Santonian–Campanian	(90)	15	115	4	55	155	15
Lower reversed Deccan traps	60–65	(63.7)A	33	98	4	51	108	11
Lower reversed Deccan traps‖	60–65	(63.7)C	32	101	—	50	113	11
Upper normal Deccan traps	60–65	(61.2)B	39	101	5	54	111	11
Upper normal Deccan traps‖	60–65	(61.2)D	35	104	—	50	114	11
Brewery limestone	base Upper Palaeocene	(57)	53	118	22	63	138	15
Sanjawi limestone§	Palaeocene–Eocene	(55)	55	123	15	63	129	15
Zam limestone§	Middle Eocene	(47)	57	129	14	61	131	15
Murree–Siwaliks¶	Miocene	(15)	72	69	17	72	69	22
B2) DSDP core data Indian Plate, also transferred to Australia								
	65–75	(70)	29	111	—	56	133	11–13
	55–65	(60)	41	120	11	54	133	11–13
	45–55	(50)	58	121	9	62	123	11–13
	35–45	(40)	67	132	12	72	136	11–13
	25–35	(30)	72	136	4	74	136	11–13
	5–22.5	(20)	79	115	—	79	113	11–13

* Stratigraphic or K–Ar ages. Mean ages used in transferring the palaeomagnetic pole position are shown in parentheses.
† Finite rotations for the Australia–Antarctica motion were according to the summary in ref. 31 for the period up to 45 Myr BP, and according to ref. 32 for reconstruction of the initial fit.

Myr BP	Lat (°N)	Long (°E)	Angle (°)
10	9.7	36.5	−6.75
21	13.8	34.6	−12.0
29	14.25	33.25	−16.0
35	12.5	34.4	−18.9
45	10.3	34.75	−23.6
>50	11.9	30.8	−30.9

Eulerian rotation poles for the India–Antarctica motions were according to the summary in ref. 3 (fixed to Antarctica).

Myr BP	Lat (°N)	Long (°E)	Angle (°)
0–32	4.9	36.4	18.3
32–53	−16.0	27.4	18.4
53–64	4.0	−3.0	15.6
64–80	−2.0	8.0	25.24
80–130	12.29	144.20	−21.85

‡ The errors (α_{95}) refer to the 95% confidence limits of the original pole position only, and do not include estimates of the errors in the finite rotations.
§ Results corrected for an inferred clockwise rotation of the Sulaiman Lobe over 50°.
‖ Mean result obtained from density distribution of palaeomagnetic pole positions[11].
¶ The Salt Range-Potwar Plateau Unit is most probably an allochthon, detached from basement[11].

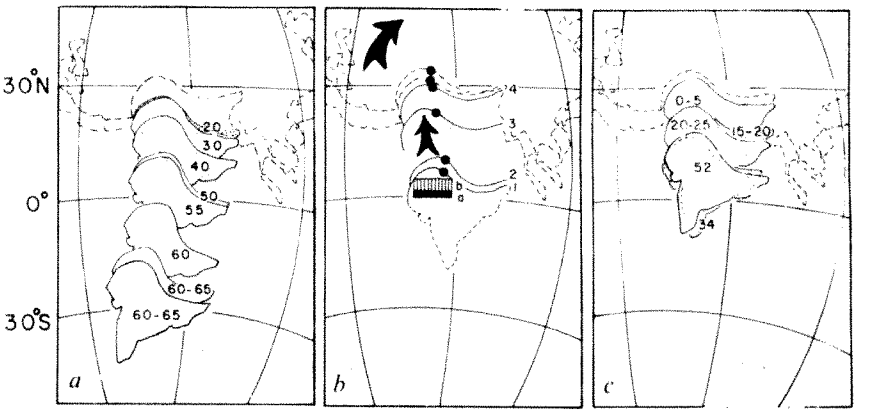


Fig. 2 Successive palaeolatitudinal positions of India according to pole positions from the Indian plate (Fig. 2*a*) and transferred pole positions from Australia (Fig. 2*c*). Note the discrepancy between India's palaeoposition according to the transferred Barrington Volcano pole position (Fig. 2*c*; 52) and the palaeopositions according to Indian data of comparable age (Fig. 2*a*; 50, 55). India's northern boundary is drawn along the Indus–Tsangpo suture zone and its western and eastern continuation. Figure 2*b* shows the palaeolatitudinal position of the Ladakh segment (large dots) of the Kohistan–Ladakh island arc north of the Indus–Tsangpo suture zone, according to four magnetisation components determined[16] in the Ladakh Intrusives (1 and 2: ~50 Myr BP and postdating initial collision of continental Indo–Pakistan with the island arc; 3 and 4: younger magnetisation components probably resulting from younger phases of the Himalayan orogenesis). For enhanced visibility the Indus–Tsangpo suture zone is shown in its present form and position relative to the Ladakh region. Zone a indicates the palaeolatitudinal position of the Upper Cretaceous Dras Flyschoids outcropping within the suture zone[11], and zone b indicates the palaeolatitudinal position of the Ladakh region according to the sampled lower Tertiary to possibly Upper Cretaceous Indus Molasse which overlies the Ladakh Intrusives[11]. The present outline of southern Asia is positioned according to mean palaeomagnetic results from Eurasia[21]. *a*: –·– 60 Myr; – – – 20 Myr. *b*: –·– 50 Myr; – – – 20 Myr. c: –·– 50 Myr; – – – present day. Linear polar projection.

crepancy probably results from inaccuracy in the pole position of the Barrington Volcano.

For a further check on discrepancies between the Indian and the Australian APWs, late Cretaceous and Tertiary pole positions from the Indian plate have been transferred to the Australian plate and the alternative APW for Australia thus obtained is shown in Fig. 3*a*. In Fig. 3*b*, this indirectly obtained APW is compared with the hitherto established late Cretaceous–Tertiary APW for Australia, as compiled before from all previously available Australian palaeomagnetic data[6,7]. This zig-zag shaped direct APW for Australia (dashed line connecting squares) is either notably diplaced from the indirectly obtained APW (Fig. 3*b*, ■ 30), does not agree with it in position or age (Fig. 3*b*, ■ 50), or is not sufficiently detailed to show the pronounced 60–65 Myr kink which is present in the indirect APW. These observed discrepancies reflect possible inaccuracies in the pole positions of respectively the Barrington Volcano (Fig. 3*b*, ● 52) and the Liverpool Volcano (Fig. 3*b*, ● 34). Apart from these discrepancies most other Tertiary pole positions for Australia (Fig. 3*b*, ●s) follow the indirect APW at an only slightly westwards displaced position.

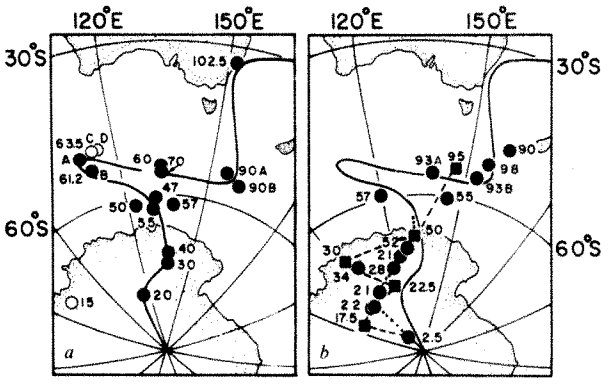


Fig. 3 *a*, The Indian APW (full line) and Indian pole positions (● and ○) transferred to Australia. *b*, Comparison of the indirectly obtained APW for Australia with an earlier compiled interpretation of Australia's APW (refs 6, 7; dashed line) and with individual Australian pole positions (●···). Compare with Fig. 1. See text for discussion and Table 1 for pole positions and confidence limits. The latter are not shown in the figures to preserve visibility. Linear polar projection. The circle (*a*, 15) indicates the transferred Indian Murree-Siwalik pole position[22]. Its quoted support[7] for the zig-zag shaped Australian APW does not seem warranted as this ill-constrained result comes from the allochthonous Salt Range–Potwar Plateau structural unit, whose rotational movement is not fully determined[11].

The indirectly obtained APW is proposed here as an alternative to the directly observed APW for Australia. The former must be considered speculative until substantiated by further (transferred) Tertiary palaeomagnetic data from continental Indo–Pakistan and corroborated by forthcoming data from Australia. Note that the zig-zag shaped direct APW for Australia is up for refinement and that previous relocations of India based on this APW can no longer be considered to be accurate. India must be positioned according to palaeomagnetic data from the Indian plate.

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An Archaean suture in Sierra Leone?

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Considerable evidence has appeared supporting plate-tectonic mechanisms for the production of Archaean metamorphic terrains[1-4]. Windley[1] has proposed models to explain both the relationship between the high grade 'gneiss' and the low grade 'granite–greenstone' terrains and the building of continental crust by collision of small continental masses. His model uses the same geological mechanisms as those typically exhibited by Phanerozoic orogenic belts, except that they were more rapid during the Archaean as a result of the fast seafloor spreading rate at that time[5,6]. I propose here that an extensive zone of mylonites, up to 10 km thick, on the coastal margin of the West African Craton[7,8] is a suture developed during the late Archaean at the site of collision of two granitic continental masses, now preserved in a partially reworked state as the Guyana Shield and the West African Craton (Fig. 1). The suture is evidence for continental coalescence in the late Archaean.

In the vicinity of Sierra Leone the mylonite is at least 400 km long and consists of severely tectonised gneisses and mylonites nearly 10 km thick[7]. The mylonite is broadly divisible into a 5-km thick zone of fine grained mylonites of both basic and granitic composition, underlain by up to 5 km of flaggy gneisses of variable composition. The gneisses and mylonites are well banded, contain deformed and granulated augen and exhibit ribbon texture. Tectonic interleaving of the rocks on each side of the mylonite zone occurred at an early stage resulting in mixed basic and granitic gneisses and mylonites. This zone of extreme deformation is sometimes not as fully developed as suggested; in southern Sierra Leone and western Liberia, for example, the mylonites seem to be poorly developed or absent.

Structurally above and to the west of the mylonite zone is a granulite facies supracrustal belt, known as the Kasila Group[7,8], which forms a linear north-west–south-east trending intensely-deformed zone adjacent to the Liberian granite–greenstone terrain of the West African Craton (Fig. 1). Approximately 8 km of fine grained basic granulites containing subordinate banded iron formation, semi-pelites and quartzites are overlain by 5 km of amphibolite, metasedimentary granulites and migmatites. The lower part of the southwesterly dipping succession is characterised by the presence of sills of coarse-grained layered leucogabbro[7].

Structurally below and to the east of the mylonite zone is the extensive granite–greenstone terrain of the West African Craton, which typically yields radiometric ages of 2,700 Myr[9]. Consisting predominantly of granodioritic gneisses and migmatites, the terrain has a structural trend discordant with the adjacent Kasila Group and mylonite zone, being generally north–south as defined by the thin synclinorial strips of amphibolite facies supracrustals.

The thickness and longitudinal extent and the structural and metamorphic characters of the Kasila Group strongly contrast with the supracrustal greenstones of the adjacent terrain. The Archaean gneisses and granulites of the Guyana Shield, although partly reworked as a result of early Proterozoic activity[9,15] tend to be of variable structural trend and like the granite–greenstone terrain of the West African Craton contrast with the essentially linear structure and extreme metamorphic grade of the Kasila Group. Although the Kasila Group is regionally discordant with the greenstone trends in the adjacent terrain, the granitic terrain immediately adjacent to it is deformed into parallellism with the high grade belt[10,11].

Some 20 km to the east of the mylonite zone is a series of disconnected exposures of low grade recumbently folded Archaean supracrustals, known as the Marampa Group[7], with a lower contact thrust against the underlying granitic rocks. They are clearly not part of a greenstone belt structure and being allochthonous, were perhaps derived as a nappe sheet from an upper level of the adjacent Kasila Group before its extreme deformation and metamorphism.

The presence of the mylonitic thrust zone, the Marampa Group klippen, the strong contrasts in stratigraphy, structural and metamorphic styles between the Kasila Group and the adjacent terrain suggests that the two major structural units were brought together tectonically. One mechanism for the production of the observed field relations could be that of a continental collision suturing orogeny similar to that envisaged for the Dalslandian[12,13], or for the Himalayas[14]. The Kasila Group supracrustals might originally have been deposited in an intracratonic or small ocean basin between two recently stabilised granitic terrains which were then driven together through the active plate-tectonic regime that characterised the late Archaean[2,5,6]. The act of complete suturing could trap basaltic crust and supracrustals between the two masses, squeezing up some at an early stage to form the Marampa Group nappe sequence, while the remainder became steadily attenuated in a vice-like situation (Fig. 1). Closure was accompanied first by plastic deformation in granulite facies conditions and then brittle deformation in retrogressive metamorphic conditions, giving rise to a thick sequence of fine grained granulite facies rocks, underlain by thrust zones of flinty material, underlain in turn by porphyroblastic flaggy gneisses. Post-collisional deformation might have thickened the zone of suturing by shortening and duplication, during which time the Kasila Group and the immediately adjacent crust were subjected to a high-temperature metamorphism due to burial in the locally thickened crust. Subsequent isostatic rebound allowed erosional processes to unroof the suture zone as a mass of material which is of a higher grade of metamorphism than the surrounding terrain.

On a much larger scale and at a deeper level of erosion, these field relations are similar to those of the root zone to the Helvetic nappes in the Ursuren Zone between the Aar and Gotthard Massifs in the Swiss Alps. The Ursuren Zone consists of a steeply dipping body of supracrustals bounded on both sides by zones of extreme deformation in which both supracrustals and the enclosing granitic material have been subjected to a very intense shear deformation. The later stages of the Alpine orogeny apparently forced the subduction of the granitic Aar massif below that of the Gotthard massif, while the sediments that were trapped between the two became intensely deformed in the

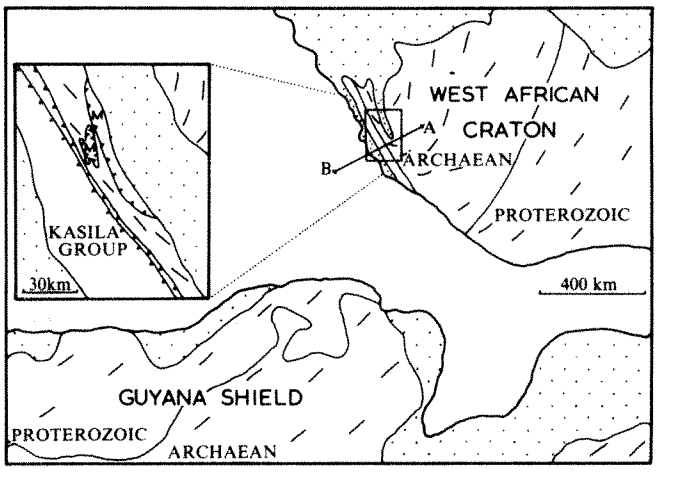


Fig. 1 Reconstruction of pre-mesozoic geology of the West African Craton and the Guyana Shield. Trend lines indicate regional structure of Archaean, much of which is partially reworked during the early Proterozoic. Post early Proterozoic cover indicated by stippling. Line A–B indicates position of idealised cross-section shown in Fig. 2. Inset shows detail of geology of western Sierra Leone, indicating the spatial relationships between the Kasila Group, the Marampa Group (M), and the thrusts and mylonites of the suture zone (thrust symbols).

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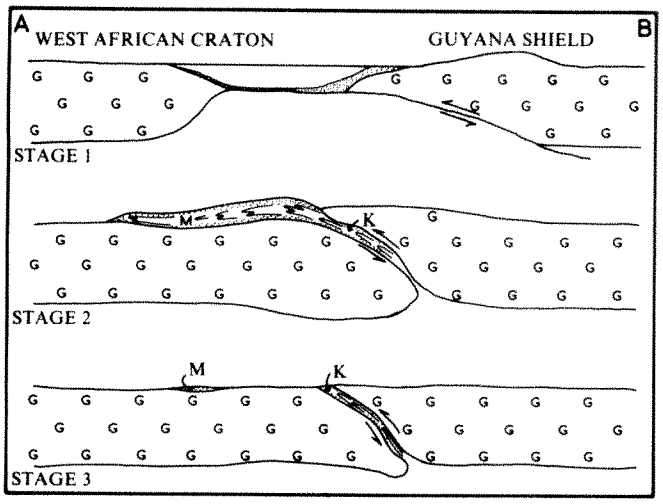


Fig. 2 Cartoon of envisaged sequence of events leading up to the formation of the Archaean suture. Stage 1, convergence of continental plates, closing of sedimentary basin floored by basaltic material. Stage 2, collision, causing plastic deformation of the Kasila Group to produce nappe sequence at high level, with granulite facies shear zones at deep level. Thickening and uprise of suture zone, overriding of the Guyana Shield onto the West African Craton. Stage 3, continued over-riding, brittle thrusting, mylonite formation in retrogressive metamorphic conditions, rapid erosion with isolation of Marampa Group as klippen.

suture zone. The granitic material on either side of the suture was also deformed, ultimately becoming schistose. If allowance has been made for the difference in scale there are strong similarities between the Cenozic Alpine and Archaean West African examples of a collisional suture.

The collision along the mylonite zone also explains the strongly developed brittle fractures in the adjacent Archaean granite–greenstone terrain of Sierra Leone[7,11]. Two main directions of fractures are obvious from the regional drainage pattern, forming a conjugate pair, one trending roughly north–south, the other east–north-east. Ideally, these two directions of fractures could be produced by a compressive stress acting from the south-west during the late Archaean suturing orogeny.

Post-Palaeozoic seafloor spreading has separated the cratonic masses which united so violently during the late Archaean, but has done so close to the line of the original suture. Kennedy[16] noticed that Pan African tectono–thermal belts tended to act as the loci for subsequent rift structures in the Mesozoic. As a result, sutures formed in early orogenic belts are susceptible to later plate activity, frequently becoming destroyed as plate-margins in orogenic belts.

If the mylonite zone and the associated high grade Kasila Group supracrustals represent a suture developed as a result of two continental masses colliding at the end of the Archaean, then it supports the opinion that primitive plate tectonic mechanisms were active in the Archaean.

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Sex ratio and niche differentiation in spinach (*Spinacia oleracea* L.)

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Studies on the population biology of dioecious plants have concentrated on deviations from the expected sex ratio of 1:1 (refs 1–4) and on sexual dimorphism[5–10]. Sexual dimorphism may imply that the two sexes have different ecologies and this has been demonstrated for a number of animals[11]. Clearly, if males and females occupy at least partially different niches, competition between members of the two sexes will be reduced and observed sex ratios may be biased by the relative carrying capacities of the two niches. Putwain and Harper[12] described differences in the seasonal patterns of growth of males and females in *Rumex acetosa* and *Rumex acetosella* and Freeman *et al.*[13] showed that the males of five dioecious plant species in northern Utah were more abundant on xeric microsites, while females were over-represented in the moister regions of each local environment. We report here that the growth of male and female plants of the annual *Spinacia oleracea* L. var. Viking is affected differently by population density, and this is reflected in the sex ratio.

The experiment was carried out in the field at Pen-y-ffridd Experimental Station at Bangor, North Wales. Seed was sown on 6 June 1978 at three densities—120, 600 and 3,000 seeds per plot, 50 × 50 cm (equivalent to distances between the seeds of about 5 cm, 2 cm and 0.9 cm, respectively). There were four replicate plots at each density in a fully randomised design.

Male plants started to flower on 22 July and the first female plants did not flower until 2 weeks later. During this 2-week period the males were very conspicuous and a casual observer could easily have recorded the population as wholly composed of males. The apparent sex ratio changed considerably after 6 August as females began to flower and branch vigorously. The populations were harvested on 17 August, when several plants had still not flowered and were spindly and trailing beneath the canopy of the flowering plants. It is unlikely that any of them would have flowered if left for longer. At harvest the male, female and unflowered plants were counted. The mean percentage dead leaves per plant (a measure of the extent of senescence) and the mean number of axillary shoots per plant were determined separately for male and female plants. The number of dead male and female plants was also recorded. Table 1 shows the proportions of sown seed that formed plants present at harvest, the proportions of plants that flowered and the sex ratios of the flowering plants. The populations showed three distinct density-dependent reactions. With increasing density the proportion of seeds giving plants still present at harvest fell from 0.546 to 0.143. Of the plants that survived to harvest the proportion failing to flower increased from 0.195% to 0.318% with increasing density. Males formed an increasing fraction of the total flowering plants as density increased. At the lowest density the sex ratio did not deviate significantly from 1 : 1 but at medium and high densities there was significant deviation in favour of male plants.

There is no reason to doubt that the sex ratio was equal among the seeds sown. Of the plants that were present at harvest a sufficient number had remained vegetative or had died to account for all the deviations in the observed sex ratios.

The mean dry weight and the mean number of axillary shoots borne by females at harvest was significantly greater than that of the males at all densities (Tables 2 and 3), reflecting their

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Table 1 Survivorship, flowering and sex ratios in populations of *Spinacia oleracea* sown at three densities

Density	Total	Total as % of seeds sown (that is, survivorship)	Failed to flower	Failed to flower as % of plants surviving	Flowered ♂	Flowered ♀	Flowered ♂ & ♀ as % of all flowering plants ♂	Flowered ♂ & ♀ as % of all flowering plants ♀	χ^2 for deviation from expected 1:1 ratio of males to females Heterogeneity (d.f. = 3)	χ^2 for deviation from expected 1:1 ratio of males to females Pooled (d.f. = 1)	
Low	262	54.6%	51	19.5%	100	111	47.4%	52.6%	1.387	0.573	NS
Medium	903	37.6%	240	26.6%	393	270	59.3%	40.7%	7.584	22.89	$P<0.001$
High	1,716	14.3%	545	31.8%	729	442	62.3%	37.7%	4.177	70.34	$P<0.001$

Table 2 Mean dry weight per plant of flowering male and female plants of *Spinacia oleracea* in populations sown at three densities

Density	Mean (g) ♂	Mean (g) ♀	s.e. of difference between the two means	Degree of freedom
Low	1.50	2.31	0.454	6
Medium	0.73	1.55	0.1612*	6
High	0.50	0.88	0.1183†	6

Significance levels: *0.01; †0.05.

Table 3 Mean number of axillary shoots developed in flowering male and female plants of *Spinacia oleracea* in populations sown at three densities

Density	Mean (g) ♂	Mean (g) ♀	s.e. of difference between the two means	Degree of freedom
Low	3.92	6.66	0.3730*	6
Medium	2.27	5.43	0.3252*	6
High	1.32	4.56	0.4827*	6

Significance level: *0.001.

continued growth, in contrast to many of the early flowering males which were already dead at the time of harvest (Table 4). The earlier senescence of the males is shown by the proportion of dead leaves borne by the plants which was higher on male plants at all densities (Table 5).

These results show both sexual dimorphism and niche separation in time in the development of the two sexes in spinach. Males are precocious in growth and flowering time but tend to senesce and die earlier than the females which continue to grow and develop axillary branches. While the earlier flowering males are dying, most of the females are setting seeds and continuing to increase the area of leaf both on the main stem and on the new axillary shoots. The continued growth of the females may be made possible by resources (for example, light) freed by the death of male plants.

Precocious flowering and senescence of the males must imply considerable wastage of pollen. However, the early death of male plants at the time when the females are setting seed may increase the reproductive vigour of the female plants. The evolution of this type of sexual niche differentiation might be explained if the dispersal of seed and pollen is highly localised so that a male is likely to favour his own progeny by dying early.

Niche separation of sexes in space or in time is probably one of the causes of observed unbalanced sex ratios in dioecious plants. In a population where one sex flowers and dies earlier than the other, the time of observation of the sex ratio becomes critical and in the present study the observed male sex ratio changed considerably over 6 weeks. A number of deviant sex ratios have been reported in *Spinacia oleracea*. Zwaan[14] found that spinach sown in a hot and dry climate produced an excess of male plants while seed sown in the cool moist climate of Holland produced nearly equal numbers of males and females. Rosa[6] commented, however, that such tests of 'place effect' were not reliable unless the different plantings came from the same seed samples. Hoffman had observed that the sex ratios of spinach appeared to vary according to the spacing of the plants (quoted in ref. 15). When plants were crowded or more or less stunted, males predominated, but with wider spacing and better growing conditions there were relatively more females (he did not give figures). When the two sexes are ecologically different and interacting competitively it is not surprising that sex ratios are altered by changes in density. Rosa[6] grew six varieties of spinach in different conditions of nutrition, spacing, shading, date of planting and mutilation. He concluded that none of these factors had any appreciable influence on sex expression but he used a lower range of densities (interplant distances of 15, 5 and 2 cm) than in the experiment reported here.

In view of the sexual niche differentiation (the Jack Sprat effect) described here for *Spinacia*, for *Rumex* species by Putwain and Harper[12] and by Freeman *et al.*[13] it is pertinent to ask how large a role such ecological differentiation may have played in the evolution and maintenance of dioecy.

There is no reason to propose that the resource demands made in the production of male gametes are the same as those involved in producing female gametes and nurturing embryos. Certainly the time sequence of resource consumption in producing male and female gametes and mothering embryos may be very different. Populations of *S. oleracea* are strongly protandrous yet because the plants are dioecious the protandry cannot be explained as an outbreeding device. If it is interpreted as one manifestation of ecological differentiation between the sexes it raises the further question of whether protandry and protogyny in hermaphrodites are really outbreeding mechanisms or whether they represent a somewhat similarly evolved non-synchrony of demand on limiting resources but occurring, in hermaphrodites, between the two gamete types within the plant or within a flower.

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Table 4 Effect of plant density on precocious death of male plants of *Spinacia oleracea*: percentage of male and female plants dead at harvest 17 August

Density	Mean (g) ♂	Mean (g) ♀	s.e. of difference between the two means	Degrees of freedom
Low	13.62	0	2.03*	6
Medium	16.93	0	4.10†	6
High	22.31	0	0.9300*	6

Significance levels: *0.001; †0.01.

Table 5 Mean percentage of leaves dead at harvest (17 August) on surviving male and female plants of *Spinacia oleracea* sown at three densities

Density	Mean (g) ♂	Mean (g) ♀	s.e. of difference between the two means	Degrees of freedom
Low	44.93	33.87	4.6122	6
Medium	59.45	34.46	4.8369*	6
High	53.56	28.29	2.5520†	6

Only leaves more than 2 cm long were recorded.
Significance levels: *0.01; † 0.001.

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Toad tadpoles associate preferentially with siblings

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Highly structured social systems can involve close kinship groups (eusocial insects[1], lion prides[2], helpers-at-the-nest in birds[3]). However, kinship generally has not been considered to be important or necessary for what seem to be merely gregarious phenomena, such as schooling behaviour[4]. In fact, Fisher first proposed kinship theory to explain the evolution of noxious taste in certain gregarious insect larvae. He reasoned that traits which at first may not be directly beneficial to the individual, such as distastefulness or warning coloration, might be selected for if they conferred advantage to siblings in the swarm[5]. Like some insect larvae, the tadpoles of many toads (genus *Bufo*) are conspicuously coloured, distasteful to predators and highly gregarious, forming densely packed schools in open areas along pond shores[6]. The tadpoles' aggregative behaviour may be important for feeding efficiency[7,8], thermoregulation[9] or predator deterrence[8], but their conspicuousness coupled with their distastefulness suggest that schooling may also serve an aposematic function. Consistent with the predictions of a kin selection model, we report here that tadpoles of the American toad (*Bufo americanus*) preferentially associate with siblings in laboratory conditions.

During May and June 1977, we collected amplectant pairs of toads from several breeding congregations. Each pair was held in the laboratory in a separate 10-l bucket, and most females oviposited in these buckets within 24 h. Each clutch of eggs was transferred to a separate aquarium, where tadpoles were reared on boiled spinach for about 45 d, until they reached developmental stage 35 (ref. 10). Tadpoles were then placed for 24 h in 0.00025% aqueous solutions of either methylene blue or neutral red. This staining technique resulted in minimal mortality and marked the translucent fins of the tadpoles for several weeks, allowing accurate identification of sibling groups by visual inspection.

To determine whether tadpoles aggregate with siblings, for each test, 25 tadpoles from one clutch were marked blue, and 25 tadpoles from another clutch were marked red. The 50 tadpoles were then released at randomised positions and allowed to acclimatise for 24 h in an indoor test pool filled to a depth of 3 cm with water. Subsequently, for test periods ranging from 4 to 7 consecutive days, the positions of all tadpoles in the pool and their clutch-identifying colours were recorded twice each day, in the morning and afternoon; thus, each test consisted of 8–14 trials. During each trial a grid of sixteen 0.05-m^2 partitioned sections was lowered into the pool and the positions of all individuals were marked on an acetate sheet fastened over the grid. The partitions limited the movement of the tadpoles during the 90-s period in which data were recorded.

The coordinate positions thus obtained were analysed by comparing the distance of each tadpole to its nearest sibling neighbour with the distance to its nearest non-sibling neighbour. If they aggregate preferentially with siblings, one would expect that tadpoles should be closer to siblings than to non-siblings. This prediction was confirmed in all six experimental tests, using different groups of tadpoles in each test (Table 1). For each test, the mean nearest-neighbour distance between siblings was significantly less than the distance between non-siblings (Fig. 1). Analysis of variance showed this sibling effect to be highly significant ($P < 0.0001$, F-test), whereas variation within experimental tests, among tests and in interactions between effects was not significant (Table 2). The among-tests effect approaches significance, perhaps due to different aggregative tendencies among tadpoles used in different tests.

The results of separate analyses of individual trials further substantiate these findings (Table 1). In the majority of the experimental trials (65%; $n = 62$), the nearest-neighbour distance between siblings was significantly less than that between non-siblings (Table 1). Although discrete aggregations of sibling groups were not always apparent, in 37% of all trials the mean coordinates of the two groups differed significantly (Table 1), indicating that the sibling groups separated out into different areas of the pool. Moreover, the tadpole groups were not simply attracted to different fixed regions of the pool, as their positions changed in sequential trials (Fig. 2). This suggests that the tendency for tadpoles to be closer to siblings than to non-siblings cannot be explained by differential responses of the two groups to possible environmental gradients in the pool.

To control for the possibility that these results were caused by either the marking or testing procedures, we carried out tests in which all 50 tadpoles were from the same clutch. Half of these individuals were stained blue and half red; all other procedures were followed precisely as in the experimental tests. In the two control tests the nearest-neighbour distances between same-coloured tadpoles were not significantly less than those between

Table 1 Group identity and analysis of separate trials within each test

Test No.	Sibling groups Red	Sibling groups Blue	No. of trials per test	No. of trials where sibling distance < non-sibling distance ($P < 0.05$)	No. of trials where mean coordinates differ ($P < 0.05$)
Experimentals					
1	B	A	8	6 (75%)	2 (25%)
2	A	C	12	7 (58%)	1 (8%)
3	A	B	14	8 (57%)	5 (36%)
4	D	A	10	8 (80%)	6 (60%)
5	E	D	10	5 (50%)	4 (40%)
6	D	E	8	6 (75%)	5 (62%)
				No. of trials where same-colour distance < different-colour distance ($P < 0.05$)	
Controls					
7	D	D	8	0 (0%)	0 (0%)
8	D	D	8	0 (0%)	1 (12%)

In the experimental tests, egg clutches were obtained from five different pairs of parents (A–E) from four separate ponds (Tompkins County, New York). Pairs A and D were from the same pond; linear distances between the ponds of origin for the other pairs of parents were: A to B, 11 km; A to C, 3.7 km; D to E, 2.6 km. In the control tests, all groups of tadpoles were obtained from the same clutch. Unlike Fig. 1, where whole tests are compared, the individual sequential trials within each test were analysed with respect to (1) whether the distance between siblings was less than that between non-siblings (Wilcoxon signed-ranks test, one-tailed[19]), and (2) whether the mean coordinates of the two sibling groups differed (Mann–Whitney U-test, two-tailed[19]). For example, test 1 consisted of eight trials, in six of which siblings were significantly closer to each other than to non-siblings. In two of the eight trials in test 1 the centres of density (=centroids) of the two groups differed. For this analysis, the centres were considered different if their positions along either the north–south or east–west axis differed significantly.

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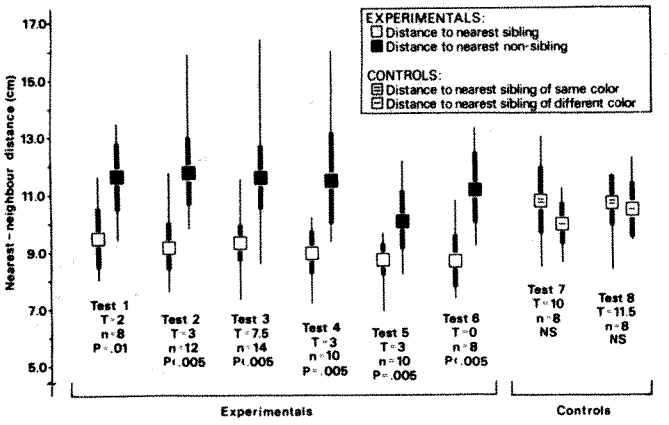


Fig. 1 Mean nearest-neighbour distance between sibling and non-sibling tadpoles (experimentals) and between siblings of the same and different colours (controls). Rectangular bars around means (symbols) represent 95% confidence intervals; vertical lines indicate ranges. Probability levels are given below the data for each test (T, n and P represent the test statistic sample size [number of pairs of trials] and level of significance, respectively). In all six experimental tests, individuals were significantly closer to siblings than to non-siblings ($P \leq 0.01$; Wilcoxon signed-ranks test). In control tests all tadpoles were siblings; same-coloured individuals were not significantly closer to each other than they were to differently coloured tadpoles ($P > 0.05$; Wilcoxon signed-ranks test).

differently coloured tadpoles (Fig. 1). Thus, the possibility that tadpoles in the experimental tests merely positioned themselves near same-coloured individuals can be discounted.

These results provide the first experimental evidence that a lower vertebrate is capable of sibling recognition. The tadpole distributions we found could arise if (1) siblings are directly attracted to one another, or (2) tadpoles avoid individuals they perceive to be non-siblings, perhaps due to aggressive encounters. We do not know what sensory modalities tadpoles use in making this discrimination, nor do we know how genetic and experiential factors effect this response. Siblings develop within a continuous jelly egg mass, and on hatching remain near the remnants of the jelly mass for several days. During these critical early developmental stages, tadpoles might 'learn' to identify siblings. Indeed, a facilitative social environment may be fundamental to the development of most normal sibling interactions[11]. Recognition of siblings might also be modifiable by experience later in life[12]. Clearly, sibling recognition need not be based solely on genetic factors.

Preferential association of tadpoles with siblings in ponds could result in the formation of sibling schools. Within schools, individuals can 'hide' from predators, thus decreasing the likelihood that they will be preyed on[13,14]. Yet to gain this advantage, individuals need not school with siblings. Indeed, the more adaptive strategy for the individual would seem to be to surround itself with non-siblings so that neither it nor its siblings are taken by a predator. However, if tadpoles associate with siblings, as our results suggest, their schooling behaviour may represent more than just 'selfish' cover-seeking. A predator that strikes a schooling individual may learn that tadpoles are distasteful, and thus it would avoid preying on other members of the school. The tadpoles' black body colour might function as a warning signal to further reinforce this 'lesson'. These traits, although not directly beneficial to the prey individual if it is eaten, may be selected for if they provide protection to related individuals in the school which are likely also to carry alleles for these altruistic traits[15]. The alarm pheromone released by injured *Bufo* tadpoles may represent another kin-selected trait, as the signalling individual is always wounded[16,17].

A sibling recognition mechanism could evolve in the following manner. In one individual, a mutation arises for distastefulness. The individual metamorphoses, survives to reproductive age, and produces young, several of which now carry the allele for distastefulness. As tadpoles associate in sibling groups for several days after hatching, because of their relative immobility, the loss of any of these distasteful individuals to a predator will benefit siblings, and thus distastefulness can spread by means of kin selection. Even if the unpalatability of a single larva is not enough to deter a predator, the cumulative noxious effect resulting from eating several larvae might be strong enough to cause future predator aversion to the school[18]. In a similar manner, aposematic coloration could spread. At this stage the benefits of distastefulness are high, but as the tadpoles begin actively swimming, and mixing with non-siblings becomes possible, the benefits become reduced or even negative. Next, a mutation arises for a sibling-association mechanism and is favoured because it prolongs the benefit of distastefulness and

Table 2 Analysis of variance for experimental tests

Source of variation	Degrees of freedom	Sum of squares	F Value	P
Sibling effect	1	77.39	44.06	<0.0001
Time effect (within tests)	7	14.49	1.15	0.36
Test effect (among tests)	5	21.97	2.44	0.06
Sibling × Test	5	5.29	0.59	0.71
Time × Test	35	71.54	1.13	0.36
Sibling × Time	7	6.88	0.55	0.79
Residual error	35	63.07	—	—

Three sources of variation and their interactions were considered. 'Sibling' refers to variation in nearest-neighbour distance dependent on whether or not that neighbour was a sibling. 'Time' refers to variation among sequential trials within single tests; only the first eight trials per test were used. 'Test' refers to variation among the six experimental tests.

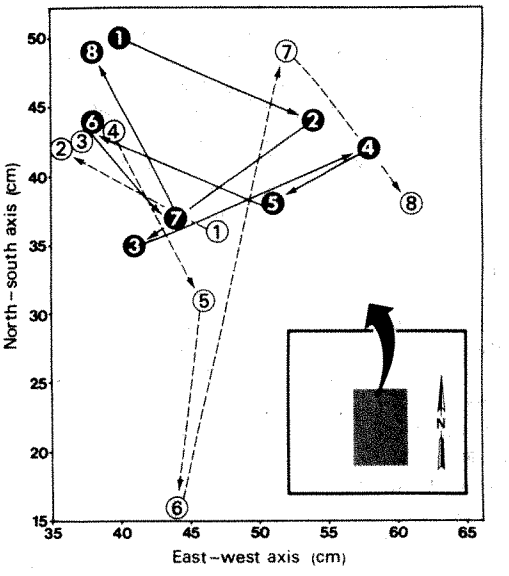


Fig. 2 Movement patterns of two sibling groups of tadpoles in a representative test. Centres of density given here are from test 6; the two sibling groups are group D (solid symbols and lines) and E (open symbols and dashed lines). The numbers inside symbols and the arrows show the sequence of trials in which the test was carried out. The two groups differed significantly in position along either the north–south or east–west axis during trials 1, 2, 3, 4 and 6 ($P < 0.05$; Mann–Whitney U-test, two-tailed). The inset shows the test pool, 88 × 96 cm, and the region of the pool in which the centres of density of the two groups were located (shaded area). A more detailed inspection of the sequential centres of density (trials 1–8) for the two sibling groups shows that each sibling group moved throughout the pool. Tadpoles were not fed during a test, nor were conditions in the pool altered. There was no evidence in this test or in the others that the separate groups preferred particular fixed regions of the pool.

aposematism into later developmental stages. Those individuals which discriminate siblings from non-siblings can preferentially associate with siblings, and thus can effectively direct their altruism towards siblings. This evolutionary scenario does not necessitate an error-free discrimination system; individuals which show any preferential sibling aggregative tendencies should have greater fitness than those which school indiscriminately with siblings and non-siblings. Although work is still in progress to identify the mechanisms by which sibling recognition is accomplished, our results are consistent with the predictions of a kin selection model.

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Ca^{2+} is essential cofactor for trypanocidal activity of normal human serum

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Normal human serum has been known to exert a cytotoxic effect on *Trypanosoma brucei* subspecies for nearly 80 yr[1]. But in spite of many attempts, no trypanocidal factor was found in human or baboon serum[2–6], until Rifkin demonstrated a high density lipoprotein (HDL) in normal human serum with trypanocidal activity[7]. The conclusion that this was the trypanocidal factor was supported by the report that serum from patients with Tangier disease, characterised by a severe deficiency of HDL, lacked trypanocidal activity[7]. We report here that Ca^{2+} is an essential cofactor for the trypanocidal activity of normal human serum, in which α_2 macroglobulin (α_2) might function as a Ca^{2+}-carrier. We further show that D-glucose, D-fructose and D-mannose can suppress the trypanocidal action of normal human serum, whereas glycerol has the opposite effect.

We used four clones of *T. brucei* subspecies. Clones of the variable antigen types (VATs) AnTat 1 and 8 were derived from a stock of *T. brucei brucei*[8]. A serum sensitive (S) and a serum resistant (R) clone of VAT ETat 2 were derived from a stock of *T. brucei rhodesiense*[8–11]. A large number of cryo-stabilates of each clone was prepared by passage through mice (female OF1 mice) and stored under liquid nitrogen[12]. Trypanosomes were collected from mice 48 h after infection, purified by passage through a DEAE-cellulose column[13], washed and resuspended in PSG (50 mM sodium phosphate buffer, *p*H 8, 36.4 mM NaCl and 83 mM glucose) or BSG (48 mM sodium borate buffer, *p*H 8, 77 mM NaCl and 83 mM glucose). Human serum samples were collected from healthy volunteers, pooled and used immediately for assays or stored at −70 °C until use. *In vitro* assay for the trypanocidal activity of serum was performed by incubating parasites (10^6 cells per ml) at 36 °C in PSG or BSG (unless otherwise noted) in the presence of human serum at the concentration specified in the figure legends. Percentage cell lysis was determined by counting the cell ghosts among at least 500 cells under a phase contrast microscope.

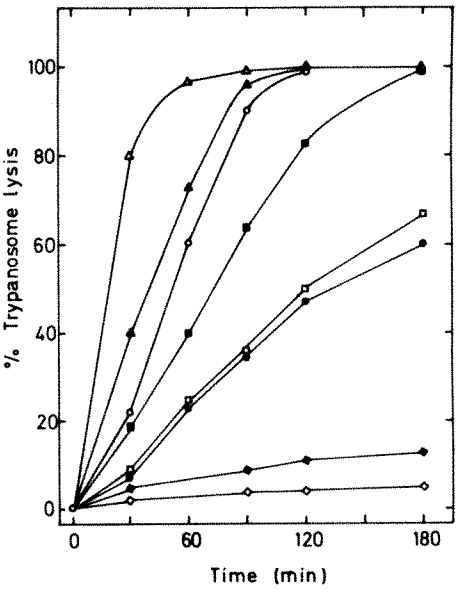


Fig. 1 Effect of dialysis, temperature and Ca^{2+} on trypanocidal activity of normal human serum. Serum was dialysed against PSG or BSG at 4 °C for 24 h with a minimal change of the volume of dialysate. The column-separated *T. b. brucei* AnTat 8 (10^6 cells per 20 μl) was incubated with 980 μl serum at 36 °C for the length indicated in the figure and cell lysis was followed as described in the text. When added, 50 μl of a stock solution of Ca^{2+} or Mg^{2+} was used in 930 μl serum to give a final concentration of 3.2 mM. ○, Undialysed serum; △, plus Ca^{2+}; ●, PSG-dialysed serum; ▲, plus Ca^{2+} or □, plus Mg^{2+}; ■, BSG-dialysed serum; ◇, heat-treated serum (60 °C, 45 min); ◆, plus Ca^{2+}.

Normal human serum, after dialysis against PSG for 24 h, had considerably less trypanocidal activity on *T. b. brucei* AnTat8 than did undialysed control serum, reaching only about 60% cell lysis even after 3 h incubation (Fig. 1). However, the addition of 3.2 mM Ca^{2+} to dialysed serum resulted in a marked restoration of the trypanosidal activity (Fig. 1). The extent of restoration was clearly dependent on the concentration (1.6–12.8 mM) of Ca^{2+} added (Fig. 2). Dialysis against PSG caused a more effective reduction of serum trypanocidal activity than did that against BSG, further implying an involvement of Ca^{2+} in this cytotoxic activity (Fig. 1). Ca^{2+} stimulated the trypanocidal activity, of not only dialysed but also undialysed serum, eliminating an initial lag period at high concentrations of Ca^{2+} (Figs 1 and 2). Another divalent cation, Mg^{2+}, had a negligible effect on the trypanocidal activity of PSG-dialysed normal serum (Fig. 1).

Furthermore, serum deprived of its trypanocidal activity by heat inactivation (45 min at 60 °C) could not be reactivated to a significant extent by the addition of 3.2 mM Ca^{2+} (Fig. 1). Thus the restoration of trypanocidal activity by Ca^{2+} requires the

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Table 1 Effect of carbohydrates on trypanocidal activity of normal human serum

Parasite	% Cell lysis in the presence of									
	Control		D-glucose		D-fructose		D-mannose		glycerol	
	PB	HS	PB	HS	PB	HS	PB	HS	PB	HS
T. b. brucei AnTat 1	100	87	1	25	2	27	0	16	0	97
T. b. brucei AnTat 8	100	96	0	35	0	39	0	26	2	100
T. b. rhodesiense ETat 2 S	100	64	0	14	2	19	0	12	0	94
T. b. rhodesiense ETat 2 R	100	2	0	0	0	0	0	0	1	34

Trypanosomes (10^6 cells per ml) were incubated at 36 °C for 2 h in 72 mM (final) phosphate buffer (PB, *p*H 8) or 50% (v/v) normal human serum (HS), containing 145 mM (final) carbohydrates as indicated in the table, except controls where 145 mM PB alone or 50% (v/v) human serum diluted with 154 mM NaCl was used.

presence of an intact trypanocidal factor in the serum, and Ca^{2+} alone cannot exert the cytotoxic effect. In addition, trypanosomes suspended in BSG containing 3.2 mM Ca^{2+} were not affected after 3 h of incubation at 36 °C (not shown).

Human α_2M is a protein ligand for Ca^{2+} (and also for other metal ions) affecting various physiological and immunological reactions[14]. Thus it was conceivable that α_2M in human serum might function at least in part, in addition to free serum Ca^{2+}, as a Ca^{2+}-carrier for a trypanocidal factor. Moreover, a fraction containing trypanocidal factor, reported by Yamaguchi[3] and Rifkin[15], contained both HDL and α_2M. To test this possibility, normal human serum was first treated with rabbit anti-α_2M antiserum, removing all immunologically detectable α_2M but leaving normal amounts of HDL and low density lipoprotein (LDL), and then dialysed against BSG for 24 h. As Fig. 2 shows, the dialysed serum had no trypanocidal activity on *T. b. brucei* AnTat 1, while the addition of 0.8 mM Ca^{2+} restored 60–70% of the activity detected in complete serum. This suggests the active involvement of α_2M in the trypanocidal reaction, possibly as an effective Ca^{2+}-carrier, whose bound Ca^{2+} cannot easily be removed by either dialysis of or the addition of EGTA or EDTA (not shown) to human serum. Rifkin's conclusion[15] that no difference in trypanocidal activity was found between serum absorbed with either rabbit anti-α_2M IgG or normal rabbit IgG can be explained by the fact that no dialysis was performed to eliminate free serum Ca^{2+} after treatment with anti α_2M IgG and sufficient Ca^{2+} (about 2 mM) was present in the *in vitro* assay system. Indeed a maximal trypanocidal activity coincided with the Sepharose 6B column fractions of LDL-free serum, where HDL and α_2M overlapped each other[15].

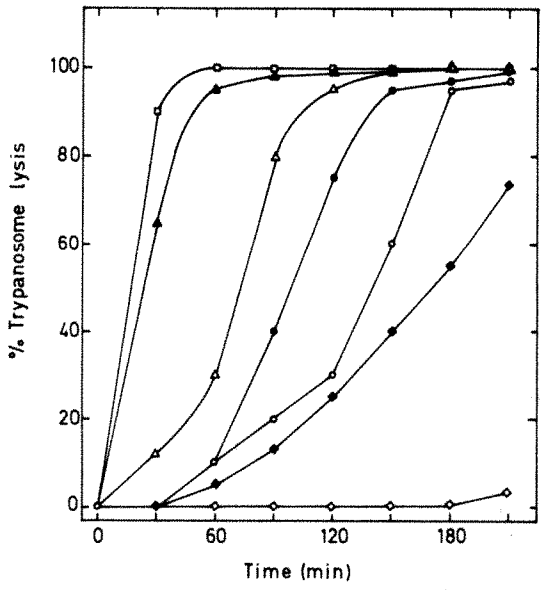


Fig. 2 Effect of Ca^{2+} and α_2M on trypanocidal activity. Serum was treated with rabbit anti-human α_2M antiserum (1.5 mg IgG per ml antiserum, Cappel Laboratories, Cochranville). Both untreated and treated (α_2M-deficient) sera were dialysed against BSG at 4 °C for 48 h. The purified *T. b. brucei* AnTat 1 (10^6 cells per 20 μl) was incubated at 36 °C with 980 μl 50% (v/v) serum or serum containing various concentrations of Ca^{2+} as indicated in the figure. Cell lysis was followed as in Fig. 1. ○, BSG-dialysed untreated serum; ●, plus 1.6 mM Ca^{2+}; △, plus 3.2 mM Ca^{2+}; ▲, plus 6.4 mM Ca^{2+} or □, 12.8 mM Ca^{2+}; ◇, BSG-dialysed treated serum; ◆, plus 0.8 mM Ca^{2+}.

The bloodstream form of *T. brucei* species can utilise D-glucose, D-fructose, D-mannose and glycerol efficiently as carbon and energy sources but depends exclusively on exogeneous carbohydrate because it can not accumulate intracellularly storage polysaccharides[16]. We examined the effect of these carbohydrates on serum trypanocidal activity towards all four *T. brucei* clones, and the results are summarised in Table 1. First, they protected the parasite suspended in 145 mM sodium phosphate buffer (*p*H 8) from lysis during 2 h of incubation at 36 °C. Trypanosomes suspended in buffer without an energy supply were lysed within 10–20 min. Second, the presence of either D-glucose, D-fructose or D-mannose markedly suppressed the trypanocidal activity of normal serum in a concentration dependent manner (not shown). Interestingly, glycerol enhanced trypanocidal activity to some extent, even with the resistant clone of *T. b. rhodesiense* ETat 2 R (Table 1). Thus a simple energy supply to trypanosomes by carbohydrate metabolism is not responsible for altering the capability of trypanocidal factor.

Gruenberg *et al.*[16] have demonstrated, by kinetic analysis of transport systems, that D-glucose and glycerol do not share the same carrier site in the bloodstream form of trypanosomes of *T. brucei*. The first event for trypanolysis by human HDL should be the binding of HDL to the membrane of trypanosome, which may be mediated by a specific surface receptor, as has been shown for serum LDL[18]. Thus it is tempting to suggest that human HDL utilises the specific carbohydrate carrier sites for its interaction with the trypanosome membrane. The carrier site for D-glucose, while involved in its transport, cannot interact efficiently with a trypanocidal factor, whereas the carrier site for glycerol enhances such an interaction. Ca^{2+} requirement for the trypanocidal activity might be mediated, among other possibilities, by its binding to the phospholipid moiety of HDL. The subsequent event involves the alteration of the membrane structure, possibly by removing lipid components as postulated by others[7]. Because subspecies of *T. brucei*, *T.b. rhodesiense* and *T.b. gambiense*, and *T.b. brucei*, cause sleeping sickness in humans and nagana in cattle, respectively, the elucidation of the mechanism by which human HDL causes trypanolysis could contribute to the development of effective prevention and treatment of trypanosomiasis.

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Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line

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A significant aspect of primary hepatic carcinoma in man is the high positive correlation of hepatocellular carcinoma with infection with hepatitis B virus (HBV)[1]. Analysis of the relationship between HBV infection and oncogenesis is difficult because natural infection with HBV is limited to man and experimental infection has been achieved only in chimpanzees and gibbons. Furthermore, because HBV has not been successfully propagated in cell culture, basic study of virus–cell interaction of the aetiological agent of one of the most widespread infections of man has been impossible[2]. Recently, however, a cell line (PLC/PRF/5) derived from a human hepatoma biopsy was described[3] which produces the HBV surface antigen (HBsAg) and so provides a tool for the experimental investigation of HBV *in vitro*. We now report the derivation and characterisation of two additional cell lines from primary liver carcinomas. In contrast to the PLC/PRF/5 cell line, these cell lines retain the capacity to synthesise many human plasma proteins, including both albumin and α-fetoprotein (AFP). One of these lines also produces HBsAg. We also present evidence that HBsAg synthesis and secretion in this cell line are correlated with the growth state of the culture. This finding is in contrast to the continuous HBsAg production found in the PLC/PRF/5 cell line.

The cell lines were established from liver tumour biopsies obtained during extended lobectomies of a 15-yr-old caucasian male from Argentina (1975) and an 8-yr-old black male from the US (1976). Tumour minces were cultured on irradiated mouse cell layers (STO). The cell lines established after these biopsies had been cultured for several months were designated Hep G2 and Hep 3B, respectively, and their morphological characteristics and epithelial cell shape are compatible with those of liver parenchymal cells. Histology of the liver biopsies revealed well differentiated hepatocellular carcinoma with a trabecular pattern. At autopsy of the second patient a portion of the lung, containing suspected metastases, was cultured. The only cells which grew from these tissue fragments were of fibroblastic morphology and are designated Hep 3L.

Supernatants from Hep 3B, Hep G2, PLC/PRF/5, Hep 3L and STO cell lines were tested for the presence of human albumin and AFP by Ouchterlony two-dimensional gel diffusion using commercially obtained antisera. Only the supernatants from the Hep G2 and Hep 3B lines formed precipitin lines which gave a reaction of identity with purified human albumin and human serum and purified human AFP and human placental cord serum, respectively. Human albumin and AFP were analysed quantitatively by radioimmunoassay inhibition testing (Fig. 1). Samples from supernatants of these same cultures were also tested for production of HBsAg, those from Hep 3B were positive whereas supernatants from Hep G2, Hep 3L and STO cultures were repeatedly negative. PLC/PRF/5 supernatants served as a positive control.

Ultrastructural examination of cells from the Hep 3B line was performed using standard electron microscopic techniques. The cells resembled previously described human and experimental liver cell carcinomas[4]. The cells contained prominent cisterns of granular endoplasmic reticulum and a moderate amount of mitochondria; stacks of annulate lamellae and aggregates of glycogen were found in the cytoplasm. No viral particles or cytoplasmic alterations indicative of HBsAg were noted in either apparently healthy or necrotising cells or in the cellular debris.

While monitoring the Hep 3B cultures during the first 1.5 yr of its growth in culture, we observed that the quantity of HBsAg in the supernatants was variable, ranging from undetectable to large amounts. In contrast, supernatants from the PLC/PRF/5 were always positive for HBsAg even within 24 h of subculturing. The same Hep 3B cultures that were variably positive for HBsAg were consistently positive for human albumin. To determine the cause of this variation we monitored the kinetics of HBsAg, AFP and albumin production in a series of replicate

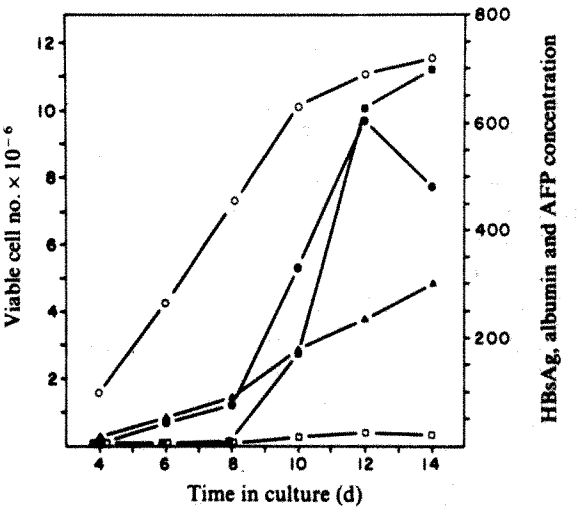


Fig. 1 T75 flasks containing confluent monolayers of Hep 3B cells were tested for HBsAg; those containing >300 ng of HBsAg were used to establish eight replicate cultures. T75 flasks containing monolayers of irradiated feeder layer (STO) cells were seeded with 10^6 viable hepatoma cells in single cell suspension (0.25% trypsin, 1 mM versene, then washed once with medium) and fed with 15 ml Eagle's minimal essential medium (AutoPow, Flow) containing 10% fetal bovine serum (Armor, heated to 56 °C for 0.5 h) and 10 mM glutamine. At days 4, 6, 8, 10, 12, 14, the supernatant fluid was removed, centrifuged and frozen, single cell suspensions prepared as above and viable cells determined by the erythrosin-B dye exclusion method. The remaining cells were washed three times in Dulbecco's modified phosphate-buffered saline, centrifuged, and frozen and thawed four times in 1 ml distilled H_2O. These total cell lysates were also frozen until assayed. Control for each assay included supernatant fluid and/or cell lysates from cultures of Hep G2, Hep 3L, PLC/PRF/5 and STO. HBsAg was quantitated using the AUSRIA II (Abbott) solid phase radioimmunoassay (RIA) kit and comparing positive values to a standard curve with purified HBsAg, determined as part of the same assay (test sensitivity of 0.65 ng ml^{-1}). Albumin and AFP were quantitatively determined in competitive inhibition RIAs by comparing values to standard curves obtained in the same tests with known concentrations of human albumin (Pentex) and AFP (test sensitivity of >20 ng ml^{-1}). Supernatants and cell lysates from STO and Hep 3L were negative for HBsAg, albumin and AFP, Hep G2 samples were negative for HBsAg, and PLC/PRF/5 samples were negative for albumin and AFP. ■, ng HBsAg in supernatant; □, in cell lysate; ▲, μg albumin; ●, μg AFP; ○, viable cell number.

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cell cultures by collecting supernatants, determining cell number and preparing cell lysates at various time points. As shown in Fig. 1, HBsAg is not detectable in either supernatants or cell lysates during the first 6 days of subculture, but can be detected by 8 days (11 ng per culture). During this interval, the cell number has increased consistently. HBsAg increased rapidly from day 8 to day 12, whereas the cell number less than doubles during this same time interval. In contrast, albumin is detectable at all time points, increasing with cell number and time in culture. AFP can be found in supernatants throughout the culture growth period, although the increase in the rate of AFP production paralleled that of HBsAg. Cell lysates quantitatively analysed for these proteins gave no evidence for a large internal pool of any of the proteins (Fig. 1, data not shown for albumin and AFP). HBsAg was found in detectable concentrations in cell lysates at the same time as in the supernatants, showing that its rapid increase in the supernatant was not due merely to release of previously synthesised protein.

Culture conditions were examined in an attempt to account for the delay in HBsAg production. To determine whether cell contact is necessary for HBsAg production, the same number of cells were plated throughout a tissue culture dish or restricted to a small portion of the dish by seeding into a plastic ring which was removed 24 h later. No effect of immediate cell contact on the kinetics of HBsAg production was found (Table 1); HBsAg was detected on the same day post-plating and the cultures containing cells in close contact did not produce more HBsAg.

Second, we examined the possibility that a component of fresh cell culture medium hindered HBsAg production or that a cellular product secreted into the medium was necessary for HBsAg production. Feeding of cultures with conditioned medium obtained either from pre-HBsAg positive cultures of Hep 3B (Table 2) or from other human and mouse cell lines (data not shown) did not result in early initiation or increased production of HBsAg, although conditioned medium was capable of supporting cell multiplication (see Table 2 legend for details of culture conditions). When the culture medium is replaced daily with fresh medium, the production of HBsAg is delayed and the quantity reduced (Table 2).

There are several possible mechanisms to explain the delayed appearance of HBsAg in the Hep 3B cell line. The simplest is that the antigen is continuously produced by each cell but that the assay system precludes detection until large amounts of HBsAg have accumulated. This is unlikely because of the sharp rise in the concentration of HBsAg as the cells approach the stationary phase of the growth cycle (Fig. 1). The lowest concentration of HBsAg antigen detectable in this system is approximately 1 ng per culture. As Hep 3B cultures containing 10^7 cells are capable of producing an average of 220 ng of HBsAg within a 24-h period, HBsAg would be detected if it was being continuously synthesised by each cell in the early cultures.

A second possibility is cell cycle control as observed in plasma protein biosynthesis by fetal rat hepatocytes[5]. These studies clearly demonstrated that the synthesis of albumin is independent of the growth state of the cells; the rate of production and release is constant throughout the growth cycle. AFP production by rat hepatocytes, on the other hand, is a function of the position of the cultures in the cell cycle. The curve (Fig. 1) for production of albumin in the human hepatoma-derived line Hep 3B reflects the results observed with rat hepatocytes; albumin is produced and accumulates in the culture supernatant fluid and a plateau is reached when the nutrient state of the culture declines. However, the kinetics of production of AFP and HBsAg by the Hep 3B cells suggests cell cycle control with production occurring when the cells stop dividing and enter the G_0 or resting phase of the cell cycle. This would explain both the appearance of HBsAg in the late log phase of culture growth (Fig. 1) and why cells repeatedly stimulated with fresh medium produce only small quantities of HBsAg. A third possible explanation is that cells in the culture differentiate or mature into terminal cells that are HBsAg producers. Experiments designed to distinguish between the last two possibilities are in progress. Interestingly, in the PLC/PRF/5 cell line, HBsAg is produced throughout the growth cycle (ref. 3 and data not shown).

Speculation on the control of HBsAg synthesis in cell lines must take into consideration the possibility that the viral sequences may be carried as episomal transcriptional units or integrated into the genome of the host. The PLC/PRF/5 genome is host-cell integrated[6] and preliminary evidence indicates that this is also the case for the Hep 3B cell line. As the site of integration may regulate the transcription of the viral information, the controlled production of HBsAg might be indicative of different integration sites in the Hep 3B and PLC/PRF/5 cell lines. Investigation of cell lines derived from human hepatomas, including those negative for HBV expression, should continue to yield insight into the relationship between HBV and human hepatocellular carcinoma.

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Table 1 Effect of cell contact on HBsAg production by Hep 3B

Culture conditions	Days after subculturing			
	6	8	10	15
Unrestricted	0*	0	4	40 (26)†
Restricted	0	0	2	28 (18.4)

0.3×10^6 cells were plated in 19-cm^2 culture dishes containing 4 ml of culture medium. Unrestricted cultures allowed growth throughout the plates; restricted cultures were established by placing the Hep 3B cells in a 3-cm^2 plastic ring which was removed 24 h later.

* ng HBsAg per culture.

† ng HBsAg per 10^6 cells.

Table 2 Effect of fresh and conditioned medium on HBsAg production by Hep 3B

Culture conditions	Days after subculturing				
	4	6	8	10	15
Conditioned medium*	0§	9	9	5 (0.4)‖	NT
Fresh medium†	0	6	27	121 (3.6)	169
Fresh medium changed daily‡	0	0	1	10 (0.4)	26

1.5×10^6 cells per 75 cm^2 per 12 ml medium subcultured in replicate flasks in the conditions listed below. Samples of supernatant fluid were recovered from the cultures on indicated days following subculture and frozen at −20 °C until assay for HBsAg. Duplicate cultures allowed computation of ng HBsAg produced per 10^6 cells on day 10. NT, Not tested; conditioned medium, although capable of supporting cell growth ($> 11 \times 10^6$ cells on day 10), could not support these cells for 15 days.

* Conditioned medium was obtained from 5-day-old cultures of Hep 3B (1.5×10^6 cells plated on day 0; 5×10^6 cells collected on day 5) which were not yet HBsAg positive; this was used to subculture Hep 3B cells. 6 ml were removed every 2 days and replaced with 5-day conditioned medium.

† 6 ml were removed every 2 days and replaced with fresh medium.

‡ Total supernatant fluid removed every day and replaced with fresh medium.

§ ng HBsAg per culture.

‖ ng HBsAg per 10^6 cells.

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Hepatitis B surface antigen produced by a human hepatoma cell line

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Infection with hepatitis B virus is associated with the appearance in the serum of a specific antigen, hepatitis B surface antigen (HBsAg), a serologically complex structure present on the coat of the double-shelled 42-nm hepatitis B virus (HBV) particle. HBsAg is also present, often in large excess, in the form of small, spherical 20–25-nm particles in the sera of infected individuals[1]. The serologically distinct core (HBcAg) or nucleocapsid of the 42-nm virus is 27 nm in diameter and contains double-stranded circular DNA with a molecular weight (MW) of $1.6–2.0 \times 10^6$, and an associated DNA polymerase[2]. A third antigen, hepatitis B e antigen (HBeAg), appears to correlate with the number of virus particles[3] and the relative degree of infectivity of surface antigen-positive sera[4], is frequently detected free in the serum and has been shown to be closely associated with the viral core[5]. Failure to propagate hepatitis B virus serially in tissue culture is a major obstacle to precise biochemical characterisation of the antigens and the development of vaccines against this important infection. The use of purified HBsAg 20–25-nm particles prepared from the sera of persistently infected donors for the immunoprophylaxis of hepatitis B is being investigated[6]. Such preparations have been shown to protect susceptible chimpanzees on challenge with HBV[7,8]. There are, however, problems associated with the collection of HBsAg positive sera from persistently infected carriers. The recent establishment of a HBsAg-producing cell line from a human hepatoma[9,10] has provided an alternative source of HBsAg particles with the added advantage that both the production and quality of the antigen may be more precisely controlled. The availability of this cell line also facilitates the study of HBV gene products by biochemical methods applicable to cultured cells, in the absence of contaminating serum proteins. In this study, the 20–25-nm HBsAg particles produced by these cells were characterised and compared with those derived from the sera of persistently infected human carriers.

A study in our laboratory of the rate of HBsAg production showed that it was minimal during logarithmic cell growth, and maximal towards the end of a 7-day maintenance period (J.A.C. *et al.* in preparation). Titres of up to 1.3 $\mu g\ ml^{-1}$ of HBsAg were obtained by incubating confluent cultures in maintenance medium for 7 days. Measurements of HBsAg, HBeAg and DNA polymerase by the most sensitive methods currently available[11] were performed on cell culture fluids, and on pellets derived by ultracentrifugation of the fluids (Table 1). Only HBsAg was detectable. Electron microscopy revealed that the predominant form was the 20–25-nm spherical particle. Pelleted HBsAg was further purified by two cycles of isopycnic banding in CsCl as described previously[12]. The buoyant density determined during this procedure was 1.19 $g\ ml^{-1}$, as found by others[13]. Material banding at this density was radiolabelled with the Bolton and Hunter reagent to a specific activity of 5 $\mu Ci\ \mu g^{-1}$ before further purification[14]. It was then subjected to centrifugation in linear gradients of Urografin[12] (Fig. 1). The majority of the radiolabel was not present in the surface antigen, as only about 10% of it was present in the region of the gradient (fractions 10–14) in which serum surface antigen was found in parallel gradients. Furthermore, when the fractions were tested

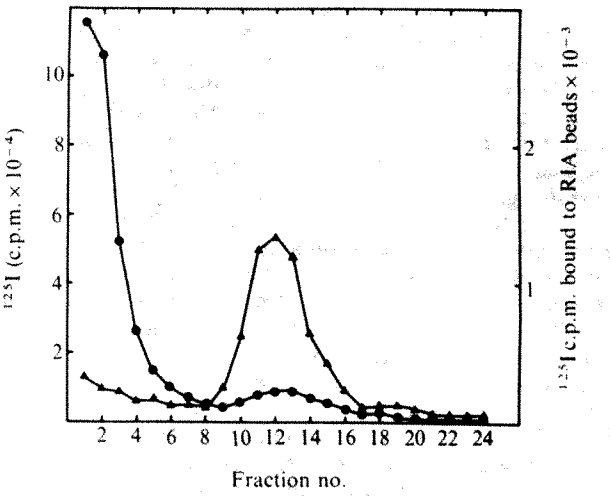


Fig. 1 Centrifugation of radioiodinated, partially purified HBsAg. Antigen was layered on to discontinuous 10–25% gradients of Urografin buffered with 0.01 Tris HCl, *p*H 7.3, and centrifuged at 52,000*g* for 18 h at 4 °C in a Beckman SW 27.1 rotor. Fractions (0.7 ml) were collected and 10-μl aliquots of each were counted in an LKB Ultrogamma counter. Fractions containing radiolabelled HBsAg were detected by direct adsorption to solid phase anti-HBsAg. Aliquots (10 μl) of each fraction were mixed with 200 μl 10% normal human serum in phosphate-buffered saline (PBS) and incubated with antibody-coated radioimmunoassay beads (AUSRIA II, Abbott) for 3 h at 40 °C. The beads were then rinsed with 10 ml of water and counted. ●, Total c.p.m.; ▲, c.p.m. bound to anti-HBsAg. Centrifugation is from left to right.

for radiolabelled HBsAg by adsorption to beads coated with hepatitis B surface antibody (anti-HBs), only fractions 10–14 were positive. These fractions were combined but attempts made to separate the antigen from Urografin by gel filtration[12] or sedimentation were unsuccessful.

In contrast to serum HBsAg, there was evidence of breakdown of the hepatoma antigen particles subjected to these treatments, as shown by failure to recover most of the radioactivity in the void volume or pellets. An alternative to rate-zonal centrifugation was therefore adopted. An affinity adsorbent column was prepared consisting of rabbit anti-HBs IgG adsorbed to protein A-Sepharose. Control experiments showed that HBsAg did not bind to protein A-Sepharose alone, or in the presence of non-immune rabbit serum. Material radiolabelled after the second CsCl step was applied to the column. After extensive washing to remove unbound radiolabel, the IgG and bound antigen were eluted with 3 M sodium thiocyanate (NaSCN). The polyacrylamide gel profiles of the radiolabelled material before affinity chromatography, and of the fraction bound by anti-HBs are shown in Fig. 2*a* and *b*, respectively. The four major polypeptides of the material before affinity chromatography had MWs of 73,000, 64,000, 54,000 and 31,500. In contrast, the MWs of the polypeptides of the affinity-purified antigen were 47,000, 34,000, 28,000 and 23,000. The latter two polypeptides co-migrated with major polypeptides of serum HBsAg run in a parallel gel. The antibody affinity step therefore allowed the recovery of the small fraction of ^{125}I-labelled material with a density of 1.19 ml^{-1} which is HBsAg reactive and uncontaminated by the other constituents of this material.

The results confirm that the HBsAg produced by the PLC/PRF/5 line is very similar in size, morphology and buoyant density to the 20–25-nm spherical particles of circulating serum HBsAg. The purification procedure developed for serum HBsAg[12] was readily adaptable to the purification of hepatoma

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HBsAg from tissue culture fluids. In spite of the fact that tissue culture supernatant contains considerably less protein than serum, our results showed that the hepatoma HBsAg required extensive purification. After two equilibrium bandings in CsCl, at least 90% of the ^{125}I-labelled protein was not HBsAg. The bulk was material which remained at the top of a Urografin rate-zonal gradient (Fig. 1) and consisted of polypeptides with MWs of 73,000, 64,000, 54,000 and 31,000 (Fig. 2). Hence, it was necessary to devise a suitable final purification step, as recovery of the antigen from the Urografin was low; also the purity and specificity of the final product before polypeptide analysis needed confirmation. Both of these aims were attained by binding of labelled HBsAg to anti-HBs IgG adsorbed to a protein A–Sepharose column. It is concluded that the final product is HBsAg for several reasons: first, it is specifically adsorbed by anti-HBS IgG; second, it contains no trace of the major polypeptides of the contaminating material; and third, it contains two polypeptides of the serum surface antigen[15–17]. Note that the hepatoma surface antigen does not contain the higher MW polypeptides frequently detected in serum HBsAg: in particular there is no polypeptide in the MW range of serum albumin which has been identified as a constituent of highly purified preparations of serum HBsAg. This observation is in agreement with our finding that the hepatoma HBsAg does not bind to Blue Sepharose CL-6B and with the observation of R. Dodd (personal communication) that the hepatoma cells produce little or no albumin. In particular, the finding of polypeptides with MWs of 28,000 and 23,000 in hepatoma HBsAg reinforces the association of these two polypeptides with HBsAg

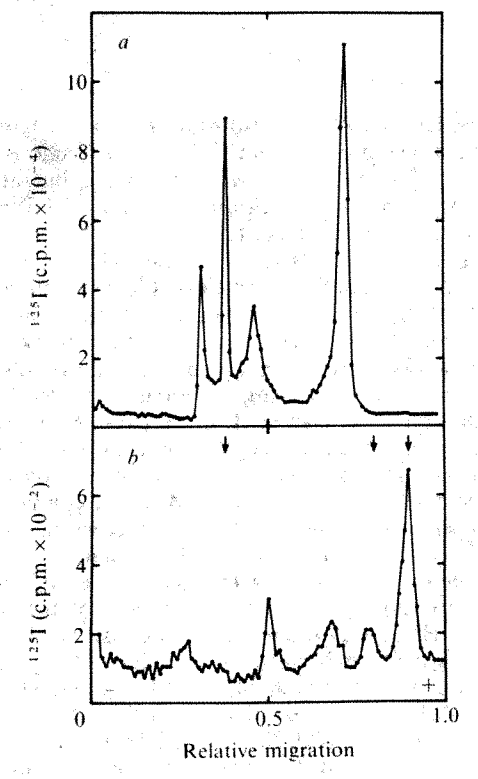


Fig. 2 SDS polyacrylamide gel electrophoresis of material radioiodinated after two isopycnic centrifugations in CsCl (*a*) and of the 3 M NaSCN eluate from the protein A–Sepharose-anti-HBsAg column (*b*). The conditions for disruption and electrophoresis in 10% cylindrical gels were as previously described[12] except that the stock acrylamide solution contained 0.8% w/v methylene bisacrylamide. Radioactivity was determined by counting 1-mm slices. The arrows indicate the positions of p64, p28 and p23 of serum HBsAg run in a parallel gel[12,15].

Table 1 Hepatitis B antigens and DNA polymerase in tissue culture fluid from the PLC/PRF/5 line

	*P/N**	
	Culture fluid	Pellet (75 × concentrate)
HBsAg	581	6,350
HBeAg	1.25	1.22
HBcAg	1.07	0.95
HBcAg after disruption	1.13	1.21
DNA polymerase	0.65	0.91

Measurements of HBsAg, HBcAg, HBeAg and DNA polymerase PLC/PRF/5 culture fluids, and in pellets derived by ultracentrifugation. Fluids were collected from confluent monolayers of cells after 7 days of incubation in maintenance medium (minimal essential medium, containing 1% fetal calf serum.) After a preliminary clarification by low-speed centrifugation, the fluids were centrifuged at 83,000*g* for 18 h in a Beckman SW27 rotor and resuspended in 1/75th the original volume. HBsAg was detected using a commercial radioimmunoassay system (AUSRIA II, Abbott) and HBeAg by a similar procedure using polystyrene beads coated with human IgG positive for anti-HBe. The same reagent was used for the preparation of the radioligand. HBcAg was measured similarly using human sera positive for anti-HBc. For this latter test, a duplicate sample was also treated with 1% NP40 and 0.1% 2-mercaptoethanol to detect the presence of HBcAg within 42-nm virus particles. DNA polymerase was measured as previously described[19].

* c.p.m. in sample/c.p.m. in control.

activity, which was previously demonstrated by Triton X-100 fractionation of serum material[15]. The other polypeptides sometimes found associated with serum HBsAg[17,18] as opposed to hepatoma HBsAg may be tightly bound serum components such as albumin[15], or components of HBsAg produced by normal rather than malignant liver cells. It is not possible to decide between these two alternatives at present.

The simple biochemical composition, and ready availability of HBsAg from the PLC/PRF/5 cell line make this line attractive as a possible source of material for intact particle or subunit vaccines for hepatitis B.

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Tumour promoter alone induces neoplastic transformation of fibroblasts from humans genetically predisposed to cancer

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Neoplastic transformation is a multi-phase process apparently caused by carcinogens and subject to the influence of promoters[1–4]. The naturally occurring phorbol esters[4–6] such as 12-O-tetradecanoyl phorbol-13-acetate (TPA) are potent tumour promoting agents. Through the use of phorbol esters a two-stage process of malignant transformation has been demonstrated in the mouse skin model[4–7] and, more recently, in cell culture systems[8–10]. Studies *in vitro* suggest that TPA reversibly inhibits terminal differentiation in most[10], but not all model systems, and that its function is presumably to increase the probability of expression of the malignant phenotype[10]. We have studied the effects of TPA on mutant human fibroblast cell strains derived from individuals with hereditary adenomatosis of the colon and rectum (ACR), an autosomal dominant trait[11]. We have previously demonstrated in these fibroblasts abnormal phenotypic expressions which often appear in transformed cells[12–17]. In these studies, we have assumed that the ACR cell exists in an 'initiated state' due to a dominant mutation and that expression of the malignant state might only require treatment with a promoting agent[18,19]. This simple experimental protocol provided a novel system for the study of cancer promotion *in vitro*[20,21]. We have now demonstrated, for the first time, the growth *in vivo* of human mutant cells exposed to TPA alone.

Subepidermoid biopsy specimens were taken from flat skin of normal appearance from ACR individuals and normal volunteers at the Memorial Sloan-Kettering Cancer Center[12]. Skin fibroblasts (SF) that grew out around the explant as a monolayer were isolated and carried in culture. The cells were grown in Earle's minimum essential medium and Earle's balanced salt solution (GIBCO) supplemented with 2 mM glutamine, 1% non-essential amino acids, penicillin (100 U ml^{-1}), streptomycin (100 $\mu g\ ml^{-1}$) and 10 mM HEPES buffer, *p*H 7.25 (EMEM) in the presence of 15% fetal calf serum (FCS). The cell strains were used between the 5th and 12th passage and were routinely checked for bacteria and mycoplasma.

The protocol for obtaining the cells used in the nude mouse inoculation experiments is shown in Fig. 1. Cells were plated in 75-cm^2 flasks at an initial density of $4 \times 10\ cm^{-2}$, and TPA (100 ng ml^{-1}) in 0.01% dimethyl sulphoxide (DMSO) was added at day 1 post-plating. Near-confluent cultures of these TPA-treated cells were continuously passaged in culture at a subculture density of $4–6 \times 10^3$ cell cm^{-2} (about 10–14 d per passage). On each passage, SF from a parallel subculture were plated in agar in the presence of 100 ng ml^{-1} of TPA and examined for colonies 4 weeks later. SF from another culture were injected bilaterally into the anterior chamber of the eye of athymic mice at a concentration of about 1×10^6 cells per 0.02 ml phosphate-buffered saline (PBS) per eye.

Table 1 describes the following cell cultures injected into the athymic mice: a DMSO-treated ACR cell culture (ME), a TPA-treated ACR cell culture (ME) which did not grow in agar during seven consecutive passages in liquid culture (Fig. 1), and a TPA-treated ACR cell culture (PF) which grew in agar (Ag I) and has been isolated from agar-growing clones on passages 2 and 4 for further studies (Fig. 1)[20,21]. As shown in Table 1, the two TPA-treated cell cultures gave rise to tumours in athymic mice, but only the PF cells were descendants of TPA-induced

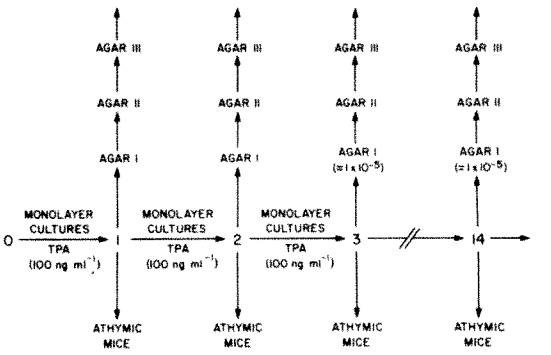


Fig. 1 The protocol for TPA-treated cells. Agar plates were prepared essentially as described elsewhere[30]. Nutrient agar (Difco), 1.84% (w/v), was melted in boiling water and equilibrated at 45 °C. It was then mixed with 2 × EMEM–30% FCS containing 200 ng ml^{-1} TPA/0.02% DMSO 1:1 (v/v) at 45 °C, and 4 ml were plated on to each of 3–5 60-mm dishes as an underlayer and allowed to firm for 15 min at room temperature. Another portion of this solution was mixed with a cell suspension of 5×10^4 cells ml^{-1} in 1 × EMEM 1:1 (v/v) containing 100 ng ml^{-1} of TPA in 0.01% of DMSO or DMSO alone. This preparation (4 ml), representing 1×10^5 cells per dish, was plated over the base layer and allowed to firm for 1 h at room temperature in a chamber irrigated with 5% CO_2. Agar colonies were scored microscopically after about 4 weeks of incubation at 37 °C in a humidified 5% CO_2 incubator. Inoculation of the nude mice (CD-1) was carried out with cells that were trypsinised, counted and washed twice with PBS to give a final cell concentration of 1×10^6 per 0.02 ml of PBS. A volume of 0.02 ml was injected under a magnifying glass bilaterally into the anterior chamber of the eye. The mice were checked every week for the appearance of tumours. These usually became apparent at 2 weeks post-injection and were removed 1–2 weeks later. Each number represents a consecutive passage.

anchorage-independent colonies (Fig. 1; Ag I). To date, we have not obtained tumours with DMSO-treated ACR cells, DMSO-treated normal cells or TPA-treated normal cells (Table 1).

At about 4 weeks post-inoculation, the optic nerve was severed and the intact eye was removed and placed in 10% formaldehyde for histological examination. A cross-section through the eye (perpendicular to the optic nerve–cornea axis) shows the occurrence of an apparently well differentiated tumour, characterised by uniformly appearing, highly basophilic, fibroblast-like cells (Fig. 2). Tumours were obtained in all mice injected with the TPA-treated ACR cells, although not always bilaterally (Table 1).

We have previously demonstrated an unusual biphasic dose–response relationship for cultured human SF to TPA[20,21], perhaps suggesting that TPA affects at least two distinct processes of cell proliferation—one which is inhibitory to cell growth, but can be saturated at relatively low concentrations, and another at the higher concentration range, which stimulates cell proliferation. Whether these results suggest the existence of at least two cell populations each of which displays a distinct type of receptor for TPA, or a single cell population with at least two types of receptor for TPA remains to be established. Consequently, in the present report we chose to study the malignant transformation of ACR cells at a high TPA concentration (100 ng ml^{-1}).

The chronic application of TPA also effects a loss of anchorage sensitivity of ACR cell cultures[20,21]. Cells selected for anchorage independence might presumably respond primarily to the growth-stimulating ability of TPA and this fraction may then conceivably be enriched by serial transfers in agar[21]. However, we found that growth in agar due to TPA occurs at a low frequency, cannot be serially sustained beyond two passages in agar (Fig. 1, Ag II) and is variable for a given human cell strain during consecutive passages in liquid culture[20,21]. For example, the human strain PF has given rise to anchorage-independent colonies at passages 2 and 4, but not at passages 1, 3, 5 and 6.

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Table 1 Effect of TPA on the tumorigenicity of ACR cells

Cell strains and treatment	Dose–response pattern to TPA*	Growth in agar	Tumours formed‖
DMSO/HC†	NA	–	0/4
TPA/HC†	Bimodal	–	0/3
DMSO/ME‡	NA	–	0/3
TPA/ME‡	Bimodal	–	4/4 (4)‖
TPA/PF§	Bimodal	+	3/3 (2)

TPA was always applied at a concentration of 100 ng ml^{-1} in 0.01% DMSO and was changed twice weekly. Controls received 0.01% of DMSO alone. TPA-treated subconfluent cultures were split at a density of $4–6 \times 10^3$ cells cm^{-2}. Colonies retrieved from agar were expanded in liquid cultures in the presence of TPA until enough cells had become available for inoculation in the nude mice. NA, not applicable.

* See refs 20, 21, and text.

† Represent SF from normal individuals. The dose–response curve of normal SF to TPA is apparently bimodal, but these cells are about 50–100-fold more sensitive to TPA than are ACR cells[20]. The latter may be due to a significant reduction in receptor sites for TPA and/or a change in the affinity of binding of TPA to its receptors in ACR cells.

‡ Represent SF from individuals with the Gardner variant of familial polyposis coli (ACR).

§ Represent SF from a clinically asymptomatic individual who is one of the F_1 progeny of an ACR pedigree and who is positive by our tests[12–22] for this trait. To date, no differences have been observed in our studies between the general case of ACR and the Gardner variant.

‖ Figures in parentheses indicate the number of mice with tumours occurring in both eyes.

This same cell strain did not grow in agar in three subsequent experiments. The human strain ME, which in this study did not grow in agar at all during seven consecutive passages, has been previously shown to grow in agar at a similar frequency to PF. However, both cell strains formed tumours all the time. The results suggest a high degree of heterogeneity in the human cells which is more apparent during selection of the TPA-induced anchorage-independent phenotype than during the process of tumour formation in athymic mice. In other words, the probability of a TPA-induced tumour is considerably higher than that of a TPA-induced anchorage independence in our system (Table 1). The results may further suggest that a loss of anchorage dependence is not necessarily a prerequisite of tumour growth and that these two processes may be modulated in parallel by separate control mechanisms[22]. In this regard, a TPA-induced permanent loss of anchorage dependence has been demonstrated with stable 'initiated' mouse epidermal cells in culture[23].

TPA has been shown to enhance the stable transformation of murine, and more recently, of human foreskin fibroblasts previously exposed to a carcinogen[8,9,24,25]. Thus, our results may indicate that the ACR mutation is a complete one for malignancy, representing an initiated state[22], and that the chronic application of TPA, in support of the two-stage 'Berenblum-hypotheses' (ref. 4) can precipitate the final oncogenic event. Alternatively, this cell mass growing *in vivo* may represent an intermediary state, similar perhaps to the TPA-induced stable papillomas in the mouse skin model[4–7] or to the clinical appearance of polyps in the colon. The latter would suggest that an additional mutation(s) is necessary for the malignant transformation of ACR cells with certain promoters acting during all phases of oncogenesis to increase the probability of expression of the malignant phenotype.

The possibility that chromosomal restructuring is associated with promotion of malignancy by TPA[26–28] in our cell system remains to be established. In this respect, an elegant analysis of lymphoma development that proposes diversity of initiation followed by convergent cytogenetic evolution has recently been enunciated[29]. The way in which the presence of TPA in ACR cells might effect a transition from the initiated state to the malignant state is now being investigated.

We thank Dr Robert A. Good for suggesting the eye as an immune-privileged site in the nude mouse system, Dr I. Bernard Weinstein for helpful discussions, Mrs C. Helson for help in the inoculation of the nude mice, and Ms P. Monaghan and Ms R. Vuolo for technical assistance. This work was supported in part by grant CA 19259 from the National Large Bowel Cancer Project, and NCI grant CA 08748 and contract N01 CP 43366.

Note added in proof: We have recently demonstrated that TPA-treated ACR cells which grew in athymic mice showed about 33% increase in cell aneuploidy (L.K. and R. Moon, unpublished). These chromosomal aberrations, if stable, may conceivably be consistent with a somatic mutation(s).

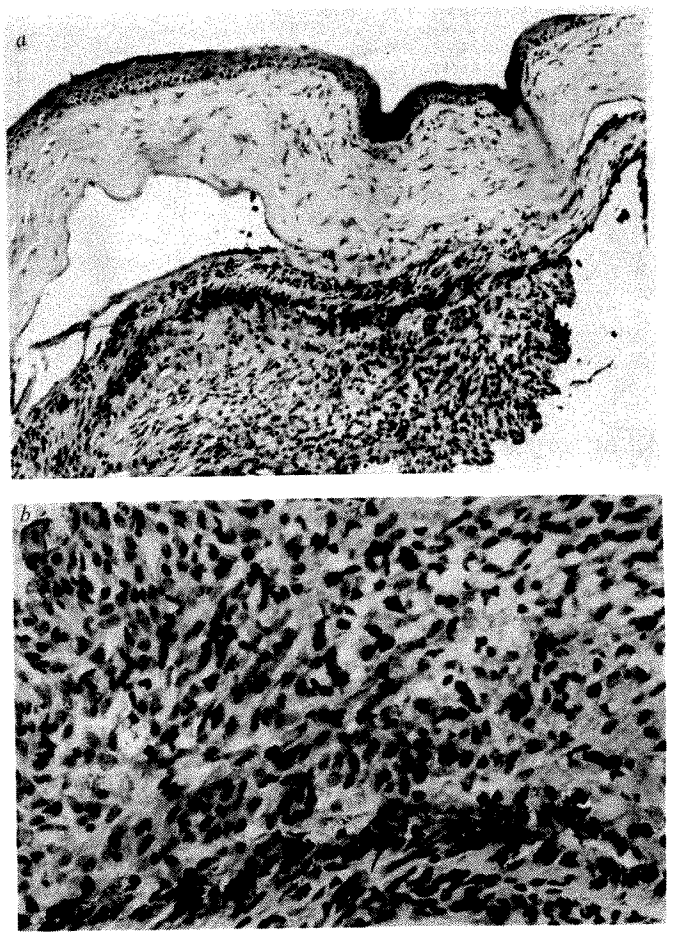


Fig. 2 A histological section of a tumour through the anterior chamber of the eye. *a*, Eyes were severed at the optic nerve and placed *in toto* in a 10% formaldehyde solution for 48 h. Cross-sections were made perpendicular to the cornea–optic nerve axis. ×90. *b*, As in *a*, magnified ×225. The pigmented particles (lower part) arose from the iris during sectioning in the periphery of the eye.

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Galactosyltransferase and membrane glycoprotein abnormality in human platelets from Tn-syndrome donors

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Early studies on the analysis of membranes isolated from the erythrocytes of Tn-patients by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a severe reduction in the staining capacity of glycophorin with the periodate-Schiff (PAS) reaction[1,2]. A low sialic acid and galactose (Gal) content of the polyagglutinable red cells was confirmed[3] while it was reported[2,4] that the abnormal red cells of Tn-patients contained little or no UDPGal: GalNAc-β-3-D-galactosyltransferase (T-transferase) activity. The glycoprotein (GP) abnormality in Tn-erythrocytes appeared to be due to incomplete synthesis of the alkali-labile oligosaccharide chains of glycophorin[2]. We now report studies on the membrane GP composition and the T-transferase activity of platelets isolated from three Tn-syndrome patients whose red cell membranes contain GP abnormalities which are typical of those found in this rare clinical condition[1,2].

There is increasing evidence that the unmodified presence of certain membrane glycoproteins is vital for the normal haemostatic functioning of blood platelets[5]. The disaccharide β-D-galactosyl (1–3)*N*-acetyl-D-galactosamine, which is the determinant of the T-antigen, has been demonstrated on the surface of human red cells[6,7] and platelets[8]. In normal human erythrocytes the T-antigen is substituted with sialic acid. The pathological exposure of the T-antigen or α-glycosidically linked *N*-acetyl-D-galactosamine (GalNAc) residues may lead to the polyagglutination of the red cells, as antibodies against the exposed antigens are present in most adult sera[9]. The polyagglutination resulting from the exposure of α-GalNAc residues forms the basis of the Tn-syndrome[10]. The frequent association between the exposure of Tn-receptors on red cell membranes and a low platelet count[10] suggested that similar modifications might also be present in the platelets of Tn-donors.

Platelets from each patient were washed and analysed by SDS-PAGE. The GP profiles of platelets isolated from patient Ba are compared with those of normal human platelets in Fig. 1. Major changes were observed in the GP pattern of the Tn platelets. The individual PAS-staining platelet glycoproteins contain different amounts of inter- and/or intramolecular disulphide bonds and exhibit characteristic changes in migration on SDS-PAGE that are dependent on the state of reduction of the disulphides[11]. Analysis of the non-reduced GP profiles clearly showed that the GP Ib/Is band was primarily affected in the Tn platelets, with its PAS-staining capacity being markedly

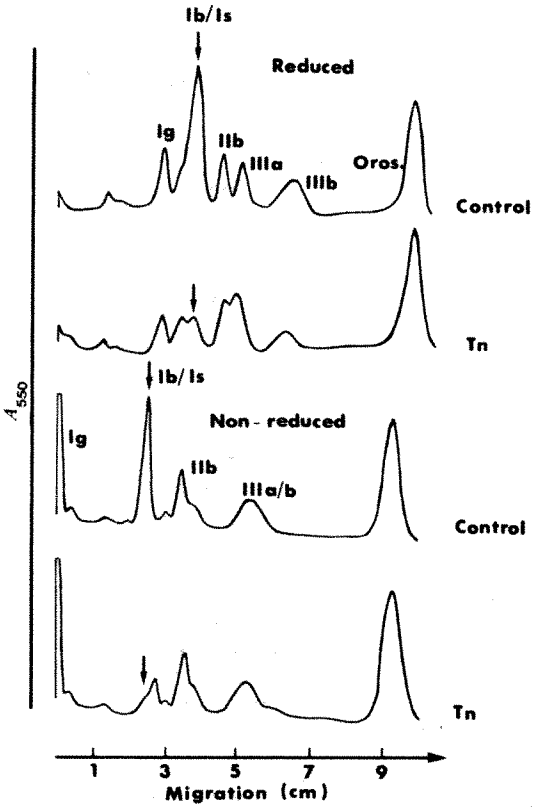


Fig. 1 A comparison of the glycoprotein profiles of normal human and Tn platelets. Washed platelets were isolated as previously described[17] and resuspended at 2×10^9 ml^{-1} in 0.01 M Tris-HCl, *p*H 7.0, containing 0.15 M NaCl, 3 mM EDTA and 5 mM *N*-ethyl maleimide. The platelets were solubilised by heating at 100 °C for 5 min following the addition of one fifth volume 12% SDS. SDS-PAGE was performed either following reduction of disulphide bonds at 37 °C for 1 h, in the presence of 5% v/v 2-mercaptoethanol (reduced samples, upper profiles) or in the absence of disulphide bond reduction (non-reduced samples, lower profiles). Electrophoresis was performed according to the procedure of Laemmli[20] using 10.5×0.5 cm polyacrylamide separating gels containing 6% acrylamide 0.2% bis (reduced samples) or 7% acrylamide 0.2% bis (non-reduced samples). Protein (400 μg) was applied to each gel together with 5 μg orosomucoid. Glycoproteins were located by the PAS reaction[17] and the gels scanned densitometrically at 550 nm. The orosomucoid acted as an internal standard for the PAS reaction. Glycoprotein profiles of platelets isolated from Tn donor Ba are illustrated. Arrows indicate the position of the Ib/Is peak.

decreased. The residual GP Ib/Is also migrated slightly faster on SDS-PAGE, suggesting that it was of a lower than normal molecular weight. The criteria used for the identification of the PAS-bands labelled in Fig. 1 are detailed elsewhere[12,13]. GP Ib, IIb, IIIa and IIIb are membrane glycoproteins. Following SDS-PAGE of unfractionated normal platelets the PAS-staining intensity of the GP Ib band is increased relative to that of the other membrane glycoproteins[12,13]. GP Is refers to the fraction of this band that has been shown to be released from the surface of washed platelets during homogenisation; it has also been termed glycocalicin[14]. GP Ib and Is may represent two fractions of a structurally similar glycoprotein[14]. GP Ig refers to an intracellular, granule-localised, glycoprotein[12].

The SDS-PAGE glycoprotein profile of the platelets of a second Tn-syndrome patient, Az, is shown in Fig. 2. In this profile the residual GP Ib/Is peak was slightly greater than was observed with the platelets of the donor Ba. We[2] have provided evidence for the presence of a dual population of erythrocytes in the blood of most Tn individuals, that is to say a subpopulation of erythrocytes with normal GP profiles and normal T-transferase activity coexisted with the pathological red cells. To

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investigate the possible presence of a dual population of platelets in Tn donors and that the abnormal GP profiles of Tn platelets was accompanied by the exposure of GalNAc residues on the platelet surface, aliquots of platelet rich plasma from patients Az and Du were separated on affinity columns of Sepharose 6MB-*Helix pomatia* lectin. This lectin specifically recognises terminal GalNAc groupings[15]. Typical SDS-PAGE glycoprotein profiles of the platelet subpopulations which either bound (HP + ve) or were not retained by the column (HP − ve) are shown in Fig. 2. The HP − ve subpopulation gave a normal GP profile, while analysis of the HP + ve platelets revealed little PAS-staining in the GP Ib/Is position.

Initial studies demonstrated the presence of T-transferase activity in lysates prepared from the platelets of normal donors (Table 1), the level of activity being independent of the ABO blood group. T-transferase activity was strongly reduced in lysates prepared from washed platelets isolated from each of the three Tn-donors studied. When the total population of platelets isolated from donor Du (with 30% of the normal level of platelet T-transferase activity) were separated into the HP − ve and HP + ve subpopulations, practically all of the T-transferase activity was located in the HP − ve platelets. As illustrated in Fig. 2 for the donor Az the HP − ve platelets had a normal GP composition, whereas little PAS-staining was observed in the GP Ib/Is position for the HP + ve platelets. Incubation of Tn-platelet lysates for 18 h at 37 °C with purified T-hapten resulted in no detectable Gal release and no evidence of an increased galactosidase activity.

Glycoprotein abnormalities have previously been reported in two congenital platelet disorders, Glanzmann's thrombasthenia and the Bernard-Soulier syndrome[5,12]. Little or no PAS-staining is observed in the GP IIb and IIIa positions following SDS-PAGE of thrombasthenic platelets, and recent results suggest severe molecular deficiencies of these membrane glycoproteins[12,16]. In the Bernard-Soulier syndrome, little or no PAS-staining is observed in the GP Ib/Is position[12,17]. As shown in Fig. 2, the HP + ve Tn platelets presented a GP profile similar to that observed with Bernard-Soulier platelets. In view of this, T-transferase activity was measured in lysates obtained from platelets isolated from a thrombasthenic and a Bernard-Soulier patient, respectively (Table 1). T-transferase activity was within the normal range in both lysates suggesting that the mechanism by which the GP abnormalities occur in thrombasthenic and Bernard-Soulier platelets is different to that found in the Tn-syndrome.

Table 1 UDPGal: GalNAc-β-3-D-galactosyltransferase (T-transferase) activity in human platelets

Donors		Blood group	Platelet count ($10^9\,l^{-1}$)	T-transferase activity* (pmol $mg^{-1}\,h^{-1}$)
Normal ($n = 7$)		A, B or O	200–400†	178 ± 28‡
Thrombasthenia BI.C		O	200	175
Bernard-Soulier Viv.		A	70	154
	Ba	O	70	13
	Az	O	120	46
Tn	Du whole platelets	O	53	58
	Du HP − ve platelets			125
	Du HP + ve platelets			17

* Measurement of the T-transferase activity was performed on platelet lysates obtained by incubating 10^9 washed platelets in 100 µl 10 mM tris-maleate buffer *p*H 7.4, containing 1% v/v Triton X-100. The assay was performed in a total volume of 100 µl containing 80 µg platelet protein, 10 µmol sodium cacodylate buffer *p*H 6.5, 1.5 µmol $MnCl_2$, 0.5 µmol *p*-nitrophenyl-2-acetamido-2-deoxy-α-D-galactopyranoside (GalNAc-α-Nph) and 0.68 nmol UDPGal ^{14}C-galactose (specific activity 280 mCi $mmol^{-1}$). After incubation for 3 h at 37 °C, the reaction product ^{14}C-Gal-β(1–3)GalNAc-α-Nph (T-hapten) was isolated by paper chromatography and quantitated by liquid scintillation counting as previously described[2].

† Normal range.

‡ Mean ± standard deviation.

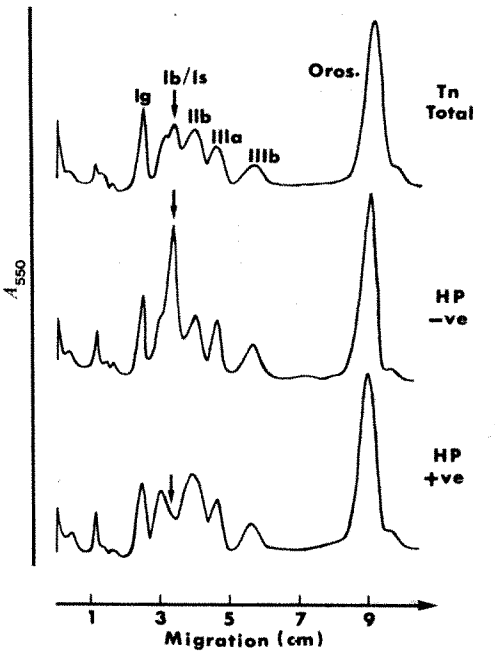


Fig. 2 Glycoprotein profiles of Tn platelet subpopulations. Platelet-rich plasma was applied to a 15 × 0.5 cm column of Sepharose 6MB-*Helix pomatia* lectin (Pharmacia) equilibrated with 0.01 M sodium phosphate, *p*H 7.2, containing 0.15 M NaCl, 50 µg ml^{-1} apyrase (Sigma) and 0.5% bovine serum albumin. Platelets not retained on the column (HP − ve) were eluted directly with the above buffer, the platelets retained on the column (HP + ve) with buffer containing 0.1 M GalNAc. The isolated subpopulations and a sample of the total platelet population were washed, solubilised with SDS, incubated with 2-mercaptoethanol and their glycoprotein composition analysed by SDS-PAGE as described for Fig. 1. Glycoprotein profiles of platelets isolated from Tn donor Az are illustrated. Arrows indicate the position of the Ib/Is peak.

The low T-transferase activity in Tn-platelets strongly suggests that the GP abnormality, as in Tn erythrocytes, arises from an incomplete synthesis of the oligosaccharide chains of the glycoprotein(s) constituting the GP Ib/Is band. This conclusion is supported by the recent detection of the T-antigen in isolated human platelet membranes[8] and the observation that binding sites for *Arachis hypogaea* lectin (which has a specificity for the T-antigen) are specifically located in the GP I position following SDS-PAGE[18]. To our knowledge, our results represent the first direct demonstration of a membrane GP abnormality common to both platelets and erythrocytes. They also point to possible similarities within the structure of platelet GP Ib/Is and glycophorin, and support the hypothesis that a somatic defect occurring in the primitive stem cells of haematopoietic tissue may be the underlying cause of the platelet and red cell abnormalities in the Tn-syndrome[19]. In common with previous reports[10] each of the Tn-donors studied possessed a circulating platelet count lower than the normal level (Table 1). Whether there is any association between the platelet GP defect and the low platelet count remains to be proved. However, the specificity of the glycoprotein defect suggests that Tn platelets provide an additional model for the investigation of the possible roles of the membrane glycoproteins in platelet function.

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Insulin binds to brain blood vessels *in vivo*

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The brain has generally been considered an insulin-independent organ, because insulin does not apparently exert a direct effect on brain glucose consumption[1]. Recently, however, insulin receptors have been detected throughout the central nervous system (CNS) of several species[2,3]. Since important insights into the functional significance of brain insulin receptors might be provided by identification of the cell type(s) possessing these receptors, we have attempted to localise them morphologically using light and electron microscope autoradiography. We report here results indicating that blood vessels throughout the CNS of the rat bind plasma insulin rapidly and with considerable specificity.

^{125}I-labelled porcine insulin (21.9×10^7 c.p.m., specific activity 134 μCi μg^{-1}) in the absence or co-injected presence of graduated amounts of unlabelled insulin, or structurally dissimilar polypeptides, ovine prolactin or bovine adrenocorticotropic hormone (ACTH), was injected intracardially into anaesthetised male rats, as previously described[4]. Five minutes after injection brains were perfused through the heart with Ringer's lactate, to wash out blood and unbound radioactivity, followed immediately by Bouin's fixative solution. Coronal 4-μm thick sections of paraffin-embedded slabs of fixed brain were stained with modified Harris' haematoxylin and eosin, and processed autoradiographically for light microscope visualisation of bound radioactivity[5].

Light microscope autoradiography showed that, in addition to prominent reactions over the circumventricular organs, which have been described elsewhere[4], ^{125}I-insulin was bound to blood vessels throughout the CNS (Fig. 1). Autoradiographic reactions occurred over brain blood vessels of all dimensions, except the large muscular blood vessesls located along the perimeter of the brain.

Although the intensity of individual blood vessel reactions did not superficially appear to correlate with the size or shape of the vessel profile, there were obvious differences in the overall regional intensity of vessel reactions among different brain regions. To achieve some quantitative appreciation of these differences, the intensity of blood vessel reactions from several regions was estimated with reference to a uniform population of vessel profiles. Thus, for grain quantitation we considered only small round nucleated vascular profiles within a narrow range of luminal diameters (13–17 μm). These microvessels were selected to provide an index of the overall intensity of blood vessel reactions for the purpose of inter-regional comparison, as they represented units of luminal surface area which were numerous and morphologically convenient to identify under the light microscope with the aid of an ocular micrometer. The total number of silver grains over 100 of these 'standardised' microvessel profiles, scored without bias, was tallied for each of several regions of brain from rats in each injection group. The average of these totals was used to estimate variations in ^{125}I-insulin binding to brain blood vessels.

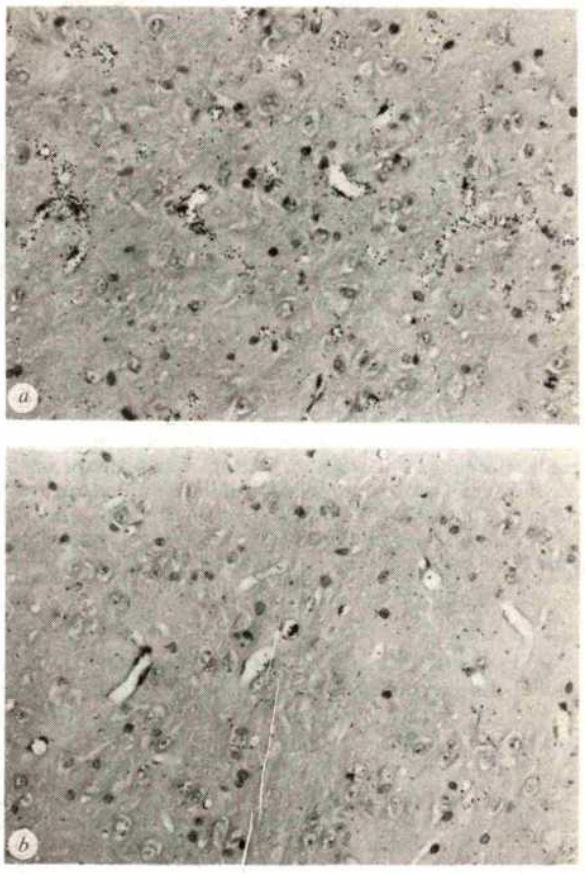


Fig. 1 The intensity of autoradiographic reactions over all neocortical blood vessels from rats injected with ^{125}I-insulin (*a*) is drastically reduced in rats co-injected with a 500-fold excess of unlabelled insulin (*b*). ×212; 2 month exposure.

In rats injected with ^{125}I-insulin alone there was marked regional variation in insulin binding to brain microvessels (Fig. 2). The most intense autoradiographic reactions occurred in neocortex, hippocampus and hypothalamus. Less binding occurred with microvessels in cerebellum and pons, and the least binding with microvessels from white matter structures, such as corpus callosum and optic chiasm. Fortuitous inclusion of the trigeminal ganglia in our autoradiographic series revealed that microvessels in this structure of the peripheral nervous system also bind plasma insulin (Fig. 2).

Quantitation of microvessel reactions was also useful in estimating the effect of unlabelled insulin and structurally unrelated polypeptides on ^{125}I-insulin binding to cerebral blood vessels. Co-injection of a 500-fold excess of unlabelled insulin reduced ^{125}I-insulin binding to brain microvessels in all regions to about the same level (Figs 1, 2). The average reduction in autoradiographic reaction was 60–70% over grey matter microvessels, but only 40–50% over white matter microvessels, because these blood vessels bound less ^{125}I-insulin when this was injected alone.

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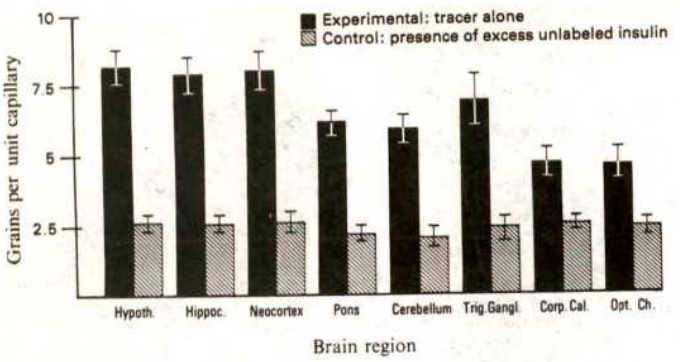


Fig. 2 Regional variations in the specific binding of ^{125}I-insulin to brain microvessels *in vivo*. The average number of silver grains (±s.e.m.) over 100 standardised microvessels (termed 'unit capillaries') is compared between experimental and control rats for several different regions of brain. Quantitation was accomplished with the aid of an 11 × 11 mm ocular micrometer disk and a Zeiss Photoscope at a magnification of ×1600. Estimates of background fog over non-radioactive brains showed 0.35 ± 0.15 grains per unit capillary.

Co-injection of ^{125}I-insulin with intermediate amounts of unlabelled insulin produced a stepwise reduction in the average reaction over neocortical microvessels, but co-injection of prolactin or ACTH had no effect (Fig. 3). These observations indicate that neocortical microvessels bind plasma insulin with a considerable degree of specificity. The specificity of the binding of insulin to cerebral blood vessels is confirmed by our recent autoradiographic studies showing that blood-borne ^{125}I-human growth hormone[6] and ^{125}I-angiotensin II (ref. 7) do not bind to cerebral vasculature.

The possible cellular location of ^{125}I-insulin binding sites in association with neocortical blood vessels was tentatively identified by means of electron microscope autoradiography of the neocortex of rats injected intracardially with ^{125}I-insulin, and killed 5 min later by perfusion–fixation as before, except that a solution of mixed aldehydes served as fixing agent. Most silver grains examined in thin section autoradiographs[8] of neocortex were situated as shown in Fig. 4 in relation to endothelium. As extensive quantitative methods[9,10] are required to determine the precise location of the bound radiolabel, our electron microscope observations must be taken as preliminary. The present data do, however, suggest that the endothelial cell may be the site of the binding interaction (M.v.H. and B.I.P., in preparation).

The results of this study demonstrate the presence of insulin-specific binding sites in association with rat brain blood vessels.

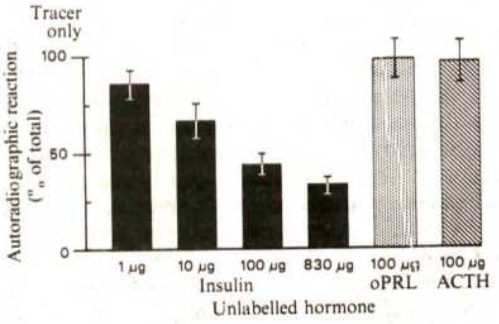


Fig. 3 Effect of unlabelled insulin and other polypeptide hormones on ^{125}I-insulin binding to neocortical microvessels. The average number of silver grains over 100 microvessels from rats injected with ^{125}I-insulin along with graduated amounts of unlabelled insulin, ovine prolactin (oPRL) or bovine ACTH is expressed as a percentage of the average number of silver grains per microvessel obtained with injection of ^{125}I-insulin alone (% of total).

All regions of brain examined showed vascular binding of insulin, although there was considerable regional variation in insulin binding capacity: the greatest insulin binding occurring with grey matter microvessels of the forebrain, and the least binding with white matter structures. It is unlikely that regional variations in insulin binding to blood vessels can be attributed to possible intrinsic differences in regional cerebral blood flow, and thus to differences in vascular accessibility to blood-borne insulin, because regional variations in autoradiographic reactions ceased when ^{125}I-insulin was co-injected with unlabelled insulin (Fig. 2). On the other hand, it is more likely that regional differences in blood vessel-associated reactions reflect regional differences in the capacity of cerebral blood vessels to bind specifically plasma insulin. This being the case, brain blood vessel binding sites for insulin may account in part for the widespread distribution of insulin receptors in the rat CNS[3].

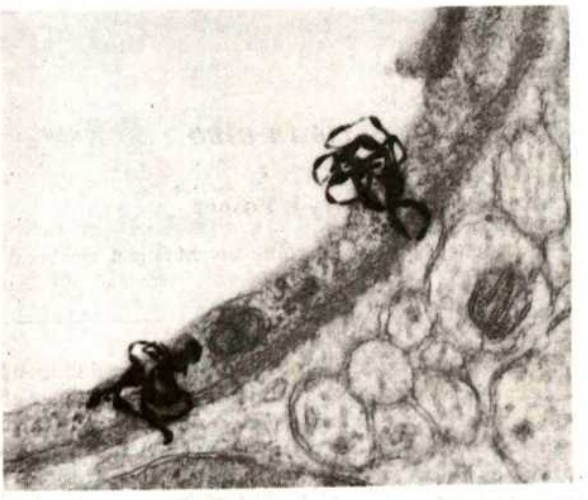


Fig. 4 Representative micrograph showing the typical location of silver grains in electron microscope autoradiographs of neocortex from a rat injected systemically with ^{125}I-insulin. The visual impression gained from examining many such grains strongly suggest that the endothelial cell is directly binding insulin. ×33,000; 4 month exposure.

Indeed, the rank order of brain regions according to microvessel binding of ^{125}I-insulin *in vivo* using autoradiographic methods (Fig. 2) is strikingly similar to the rank order of regions according to receptor content, as determined by classical direct binding studies[3].

Are brain blood vessels targets for the direct action of insulin? The specificity of insulin binding to brain blood vessels argues for these vessels having the recognition capacity concomitant with an ability to respond to plasma insulin. However, it is generally assumed that insulin has no direct effect of any kind on brain activity because it does not alter the rates of glucose transport across the blood–brain barrier[1]. The intriguing studies of Arieff *et al.*[11], on the other hand, suggest that insulin may directly stimulate potassium uptake by the brain *in vivo*, and that a direct action of insulin on brain electrolyte balance exerted at the level of the endothelial cell may contribute to the onset of insulin-induced seizures. Although by no means supporting this concept, the present localisation of insulin binding sites to cerebral blood vessels does raise the possibility that insulin may exert a generalised effect on brain activity by a direct action on aspects of blood–brain barrier function perhaps unrelated to glucose uptake.

In view of the possible relevance of the present observations to the general vascular complications of diabetes, it is important to determine whether insulin-receptor blood vessels exist outside the CNS. Bar *et al.*[12] recently identified genuine insulin receptors on endothelium isolated from human umbilical vein.

Autoradiographic studies similar to the ones presented here should be carried out to determine whether vascular beds in other tissues possess insulin binding sites.

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Localisation of phencyclidine-induced changes in brain energy metabolism

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The abuse of phencyclidine [1(1-phencyclohexyl)piperidine, PCP], commonly referred to as angel dust or hog, is rapidly reaching epidemic proportions. PCP users often appear violent and increases in PCP-implicated homicides and suicides have been reported[1]. In animal studies PCP has been demonstrated in brain up to 48 h after administration, long after blood levels become undetectable[2]. However, there is little further information on the distribution of PCP within the central nervous system with regard to the possible sites of action. Recently, Sokoloff and associates[3,4] described a new technique which can be used to visualise possible sites of drug action. The technique is based on the premise that neuronal activity is closely related to energy metabolism. Therefore, by directly monitoring 2-deoxy-D-glucose consumption before and after a pharmacological stimulus, we can obtain autoradiographic evidence of changes in neuronal activity in discrete areas of brain as a response to that stimulus. Using this procedure, we now report that PCP causes dramatic changes in glucose metabolism in very specific regions of the rat brain.

Female Sprague–Dawley rats (250 g) were used in all experiments. In pilot studies the peak dose/response of PCP as tested behaviourally in a rotometer was 5 mg per kg 40 min after intraperitoneal administration (S.D.G. *et al.*, in preparation). Thus, 40 min after PCP injection, animals were injected with 25 μCi of ^{14}C-2-deoxy-D-glucose (specific activity 53 mCi mmol^{-1}, NEN), administered through a cannula implanted in the right jugular vein 24 h before injection[5]. The conscious, freely moving animals were killed 30 min later by decapitation and processed for autoradiography[3].

The autoradiograms presented a striking effect of PCP on local cerebral glucose metabolism. Both increases and decreases were markedly apparent as determined by comparing the densities of the X-ray autoradiograms. The major sites of increased metabolism were components of the limbic lobe. Specifically, glucose consumption was increased in the molecular layer of the regio superior of the hippocampus. Both dorsal and ventral levels of the hippocampus were affected, although the greatest increases seemed to occur at posterior levels. This line of increased activity continued dorsally through the subicular cortex and into the retrosplenial cortex (posterior cingulate gyrus) (Fig. 1*d*). The anterior cingulate gyrus (Fig. 1*e*) and anteroventral nucleus of the thalamus demonstrated similar increases. Smaller increases in glucose utilisation were seen in a few non-limbic structures such as substantia nigra. An unusual finding was that the inferior colliculus, which normally has the highest energy demands of all brain areas[3], showed a marked loss of metabolic activity (Fig. 1*f*).

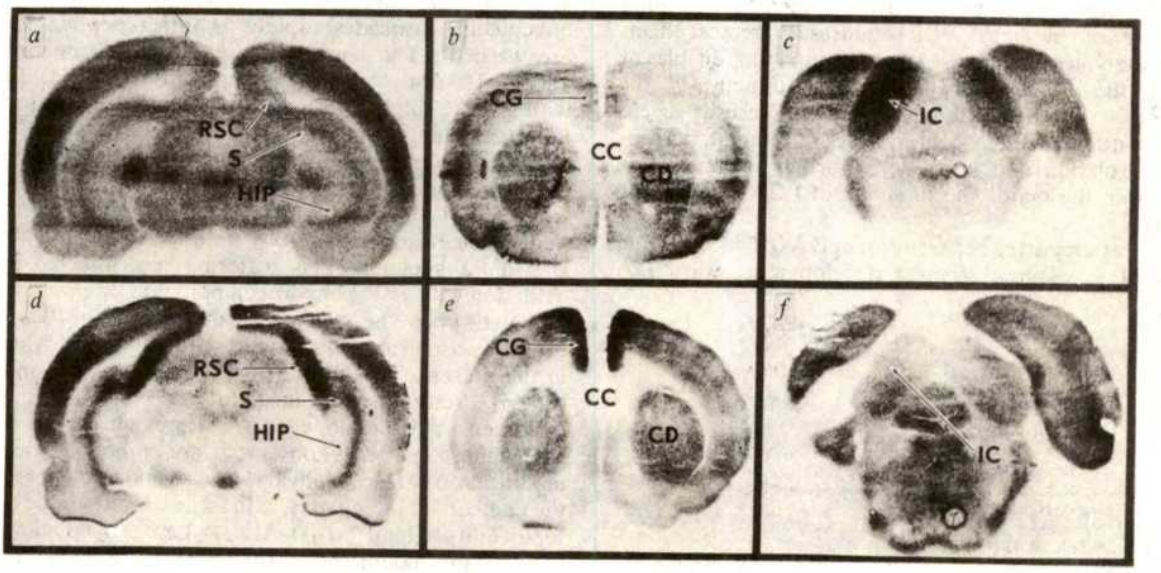


Fig. 1 Autoradiograms taken from three different levels in untreated (*a–c*) and PCP-treated (*d–f*) animals. Note line of increased intensity (glucose metabolism) in hippocampus (HIP), subiculum (S) and retrosplenial (posterior cingulate) cortex (RSC) which continues rostrally (*e*) into anterior cingulate cortex (CG). At the level of the midbrain (*c*, *f*) there is a dramatic decrease in intensity in the inferior colliculus (IC). CC, corpus callosum; CD, caudoputamen.

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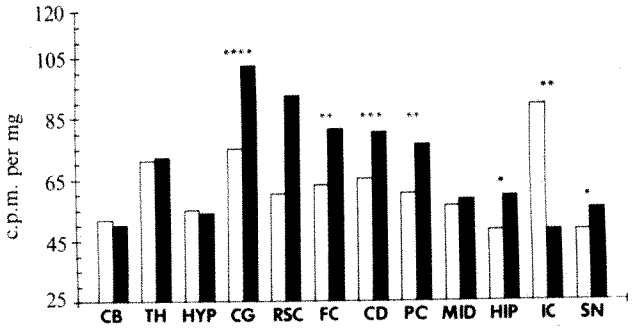


Fig. 2 A comparison of ^{3}H-deoxyglucose uptake in untreated ($n = 7$) animals (open bars) and in animals receiving 5 mg per kg PCP ($n = 7$) 40 min before injection of ^{3}H-deoxyglucose (solid bars). CB, cerebellum; TH, thalamus; HYP, hypothalamus; CG, anterior cingulate gyrus; FC, frontal cortex; CD, caudeputamen; PC, posterior cortex; MID, midbrain; IC, inferior colliculus; SN, substrata nigra. MID includes IC and SN, but was further dissected into IC and SN in three additional animals. The RSC was removed from only two animals. *Significantly different from normal in paired t-test, $P<0.05$; $^{**}P<0.02$; $^{***}P<0.01$; $^{****}P<0.005$.

In an attempt to quantify the changes in energy metabolism without using a densitometer, we repeated our experiments substituting a tritium-labelled isotope for the ^{14}C (50-μl injections with specific activity 40 Ci mmol^{-1}). After death, the brains were removed, weighed, dissected into 12 regions and homogenised in 1 ml sodium phosphate buffer, pH 7.4. 100 μl aliquots (in quadruplicate) were removed, dissolved in 10 ml of NEN scintillation cocktail and 1 ml distilled water, and counted in a Beckman LS9000 scintillation counter (30% efficiency). The results of these experiments completely supported the autoradiographic data (Fig. 2); that is, the cingulate gyrus reflected the largest increase in glucose consumption (37%) whereas the inferior colliculus had the largest decrease (54%). In addition, increases in glucose consumption which were not apparent on visual inspection were found in both the frontal cortex and caudoputamen.

The present study clearly demonstrates a marked effect of PCP on the limbic system. Papez originally proposed an emotional circuit linking the cingulate gyrus, hippocampus and anterior thalamus[6]. Later studies have included the subicular cortex in these connections[7,8]. PCP produces its greatest effects on energy metabolism in all these structures and thus enables us to visualise the functional Papez circuit directly in the autoradiograms. The fact that in humans PCP causes severe emotional disorders including disturbances of thought, perception, mood, isolation and hostility[1] is further evidence that within the brain the major site of action of PCP is within the limbic system.

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Role of disulphide and sulphydryl groups in clustering of enkephalin receptors in neuroblastoma cells

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Image intensified fluorescence microscopy has been very useful in visualising the patterns and mobility of receptors for epidermal growth factor (EGF), insulin and α_2-macroglobulin in intact cells[1,2]. Recently, we have synthesised a bioactive derivative of enkephalin[3], Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine, and used it for the microscopic visualisation and localisation of opiate (enkephalin) receptors in neuroblastoma cells[4]. In contrast to the case of polypeptide hormones[1,2], where fluorescently labelled receptors are initially distributed uniformly and quickly form patches which are subsequently internalised[1,2], the enkephalin-labelled receptor patches in neuroblastoma cells appear only slowly and are not internalised[4]. Sulphydryl groups are involved in the binding of opiates to brain membranes and may affect differentially the binding of agonists and antagonists[5,6]. Also, sulphydryl and disulphide groups are involved in the binding sites of adrenergic, cholinergic and muscarinic receptors[7–11]. These observations prompted us to examine the effects of disulphide and sulphydryl reagents on the clustering of opiate (enkephalin) receptors in N4TG1 neuroblastoma cells. We report here that in these cells there are reactive sulphydryl and disulphide groups which are essential for cluster formation (but not binding), and that a sulphydryl–disulphide exchange reaction may be involved in this process. In addition, the sulphydryl reagents seem to dissociate the two steps of binding and cluster formation, and thus provide a tool for studying the pharmacological importance of receptor clustering.

When N4TG1 neuroblastoma cells are incubated with 10^{-8} M of a rhodamine-labelled enkephalin derivative (30–40 min at 25 °C), many patch-type areas of bright fluorescence, spreading over the cell surface, are detected (Fig. 1*a*). These patches do not internalise[4] with longer periods of incubation at 25 °C or 37 °C. Furthermore, most of the fluorescent patches disappear and more than 95% of the cell-bound radioactively labelled enkephalin dissociates rapidly as intact peptide if the cells are washed[4]. The clusters are not seen in the presence of 10^{-6} M [D-Ala^2, D-Leu^5] enkephalin (Fig. 1*b*). Incubation with specific sulphydryl reagents (10^{-3} M iodoacetate, 10^{-4} M iodoacetamide, 10^{-5} M *N*-ethylmaleimide or 10^{-3} M 5,5′-dithiobis(2-nitrobenzoic acid)) in conditions identical to those used for binding assays (Table 1) result in the uniform distribution of the fluorescently labelled receptors (Fig. 1*c*); fluorescence can be displaced (inhibited) by 10^{-6} M [D-Ala^2, D-Leu^5] enkephalin. This pattern is not affected by further oxidation with 10^{-4} M hydrogen peroxide. Identical results are obtained when the cells are pretreated with 10^{-5} M dithiothreitol, washed and treated with sulphydryl-modifying reagent, in the presence or absence of subsequent oxidation (data not shown). Pretreatment or incubation in the presence of 10^{-4} M hydrogen peroxide alone does not prevent cluster formation. The diffuse patterns observed are probably due to the modification of free sulphydryl groups and not to a reduction in the number of binding sites, as in these conditions the cells retain their ability to bind ^{125}I-[D-Ala^2, D-Leu^5] enkephalin (Table 1).

When the rhodamine–enkephalin derivative is added to the cells in the presence of 10^{-5} M dithiothreitol, no patches are seen and the fluorescence is distributed evenly over the cell surface (Fig. 1*d*), even after prolonged (2 h) incubation at 25 °C. This diffuse pattern (10^{-5} M dithiothreitol, 40 min) is quickly

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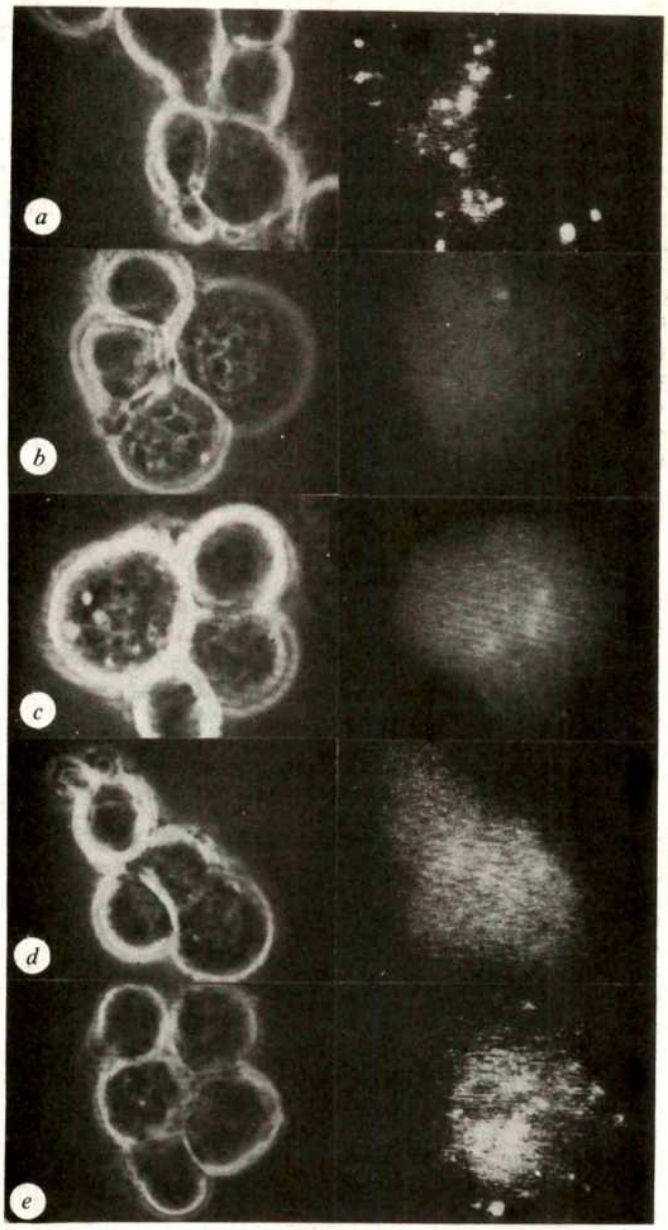


Fig. 1 Fluorescent visualisation of rhodamine–enkephalin binding to N4TG1 cells. On the left, phase micrographs ×865 on the right, fluorescence micrographs of the same field. *a*, The cells were incubated for 40 min at 25 °C with 10^{-8} M rhodamine derivative; *b*, incubation as in *a* but in the presence of 10^{-6} M [D-Ala^2, D-Leu^5] enkephalin; *c*, incubation (identical conditions as for binding assays in Table 1) with 10^{-3} M iodoacetate; *d*, incubation with 10^{-5} M dithiothreitol; *e*, pretreatment with 10^{-5} M dithiothreitol (30 min) followed by addition (for 10 min) of 10^{-4} M H_2O_2. Identical results to that shown in *c* were obtained with 10^{-4} M iodoacetamide, 10^{-5} M *N*-ethylmaleimide and 10^{-3} M 5,5′-dithiobis(2-nitrobenzoic acid). The fluorescence was recorded on videotape using a silicon intensified target television camera[4], and photographs were taken from the video monitor. The cells were not fixed for these studies.

(10–15 min) modified to one of clustering by oxidation with 10^{-4} M hydrogen peroxide (after 10 min, Fig. 1*e*; after 15 min it is similar to that seen in Fig. 1*a*). Clusters reappear spontaneously but slowly (about 75 min) when the cells are pretreated for 20 min with 10^{-5} M dithiothreitol, washed and incubated at 25 °C in the presence of the fluorescent peptide. However, the size, brightness and number of clusters are reduced (data not shown) compared with the control (Fig. 1*a*) or with hydrogen peroxide-induced oxidation (Fig. 1*d*).

The clusters, once formed, cannot be dispersed by the subsequent addition of 10^{-3} M iodoacetate, 10^{-4} M iodoacetamide or 10^{-3} M dithiothreitol, suggesting that the sulphydryl and disulphide groups are protected when the hormone is bound to the receptor and clusters are formed. The clusters can be dispersed by the addition of 100 mM sodium, which also markedly reduces the binding of the peptide, or by the simultaneous addition of 10^{-3} M dithiothreitol and 100 mM sodium. These results are consistent with previous studies with brain membrane preparations[5,6] which suggest that sulphydryl groups are associated with the opiate receptor and that binding of the hormone reduces their reactivity with sulphydryl-alkylating reagents.

Table 1 Effect of sulphydryl, disulphide and miscellaneous reagents on ^{125}I-[D-Ala^2, D-Leu^5] enkephalin binding and clustering in N4TG1 cells

Reagent	^{125}I-[D-Ala^2, D-Leu^5]-enkephalin specific binding (% control)	Hormone–receptor complex pattern
Iodoacetate		
0.1 mM	100	Few clusters
1 mM	89	Uniform distribution
10 mM	83	Uniform distribution
Iodoacetamide		
0.01 mM	95	Few clusters
0.1 mM	89	Uniform distribution
1 mM	87	Uniform distribution
N-ethylmaleimide		
0.01 mM	95	Uniform distribution
0.1 mM	50	Uniform distribution
1 mM	28	—
Dithiothreitol		
0.01 mM	94	Uniform distribution
0.1 mM	83	Uniform distribution
1 mM	77	Uniform distribution
Hydrogen peroxide		
0.1 mM	100	Clusters
Dithiothreitol (0.01 mM, 30 min) +hydrogen peroxide (0.1 mM)	95	Clusters
5,5′-Dithiobis(2-nitrobenzoic acid)		
0.1 mM	85	Clusters
1 mM	65	Uniform distribution
Sodium azide		
1 mM	82	Clusters
10 mM	67	Clusters
Dinitrophenol		
0.5 mM	100	Clusters
1 mM	98	Clusters
Methylamine hydrochloride		
1 mM	81	Clusters
10 mM	76	Small reduction in the number of clusters
30 mM	52	Few clusters

N4TG1 neuroblastoma cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. At confluence, the cells were removed from the flask by incubation with 1 mM EDTA for 5 min at 37 °C. The detached cells were washed three times with 25 mM Tris-HCl, *p*H 7.7, containing 0.25 M sucrose, 2 mM $MgCl_2$ and 5 mM glucose, and finally suspended in the same buffer. Fluorescence microscopy was done as described in Fig. 1 legend. Binding assays: the radioactive enkephalin (0.5 nM, specific activity 2 Ci μmol^{-1}) was incubated with different concentrations of the reagent at 25 °C for 60 min in a final volume of 0.25 ml containing N4TG1 cells (4×10^6 cells ml^{-1}). When iodoacetate and iodoacetamide were tested the cells were pretreated with the reagent for 15 min at 25 °C, washed by centrifugation and the binding measured by filtration under vacuum through Whatman GF/C filters as described previously[14–16]. Specific binding represents the bound radioactivity which can be competed for by 10^{-6} M unlabelled [D-Ala^2, D-Leu^5] enkephalin; each value is the mean of duplicate incubations which varied by less than 5%. All reagents were dissolved immediately before use.

To investigate further the mechanisms involved in clustering we examined the effects of sodium azide (1 and 10 mM) and dinitrophenol (0.5 and 1 mM), as shown in Table 1. The results suggest that the formation of clusters, in these conditions, is independent of the generation of metabolic energy. The effects of methylamine on clustering were also examined as this reagent has been found to inhibit the clustering of α_2-macroglobulin and EGF on the fibroblast cell surface[12]. It was suggested[12] that certain amines may act by increasing the intracellular *p*H, thus possibly interfering with protein–protein interactions necessary for clustering, or alternatively by inhibiting a calcium-requiring transglutaminase enzyme which may be involved in cluster formation. No inhibition of clustering is seen with 1 mM methylamine whereas with 10 mM there is a small reduction in the number of clusters. At 30 mM methylamine only a few clusters are observed but the binding is substantially reduced (52%). Moreover, in contrast to the observations with α_2-macroglobulin and EGF, in which clusters are not formed in the absence of extracellular calcium, the clusters in neuroblastoma cells are formed in the absence or presence of calcium or EDTA[4].

It is interesting that the nature of the binding and surface distribution of the neuromodulator, enkephalin, is quite different from that of polypeptide hormones with respect to internalisation[1,2,4,13] and the effects of sodium and calcium[4]. Our data strongly suggest that both sulphydryl and disulphide groups are specifically involved in the molecular processes involved in the aggregation of ligand–receptor complexes in neuroblastoma cells. Whether the clusters are formed or stabilised by simple sulphydryl–disulphide interchange reactions, or whether these functional groups are involved in some other capacity such as transglutaminase[12], remains to be established. However, prevention of cluster formation without affecting receptor binding may provide a tool for studying the roles of receptor occupancy and subsequent clustering in such biological effects as opiate tolerance and addiction.

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Relationship between crystallin mRNA expression in retina cells and their capacity to re-differentiate into lens cells

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Differentiating vertebrate embryo tissues pass through a stage when they have the potential for several pathways of differentiation, some of them revealed only by experimental manoeuvres. As differentiation proceeds, the potential for alternative pathways becomes progressively restricted and is eventually lost. We have investigated the molecular basis for this phenomenon, using the chick embryo retina, which has a potential for differentiation into lens cells. This potential requires special conditions for its manifestation; it declines during development and is finally lost before hatching. Lens protein (crystallin) mRNA is expressed in early embryo retina: its levels decline in parallel with the decline in lens-forming potential. There is also evidence of an ontogenic change in post-transcriptional regulation. Such a relationship between heterologous mRNA and heterologous differentiation potential may be of general application.

In the vertebrate eye the neural retina (NR) and the pigmented epithelium (PE) of the retina are of different developmental origin to the lens, but embryonic cells from both tissues are able to redirect their differentiation in cell culture into lens fibre cells, with characteristic ultrastructure, and containing the characteristic lens proteins, the crystallins. This phenomenon has been termed transdifferentiation: it has been found in all vertebrates so far tested[1–3]. In the chick, which has been the most intensively studied example, the expression of the capacity for transdifferentiation is regulable by cell culture conditions[1,3–5] and depends on the age of the embryo, transdifferentiation being more frequent and more rapid from retina of early as compared to later embryos, while the relative balance of the crystallins synthesised is also affected[6–8]. The three classes of crystallins in birds are the α-, β-, and σ-crystallins. Lentoids (structures composed of lens fibre cells) have the highest proportions of σ-crystallins when derived from retinas of earlier embryonic stages (Table 1).

We have found that crystallin mRNAs are expressed in 8-day embryo neural retina (NR) at levels of about 0.025% and in 8-day embryo retinal pigmented epithelium (PE) at levels of about 0.04% of those found in 1-day-old lens, but embryo muscle and headless embryos contain no detectable crystallin mRNAs as measured by hybridisation to crystallin-specific cDNA[9]. During the process of transdifferentiation into lens from 8-day embryo NR or PE, the levels of crystallin mRNA rise, and in terminally transdifferentiated NR cultures, reach between 14% and 17% of the levels found in 1-day-old lens. Terminally transdifferentiated PE cultures reach 1.25% of the level in day old lens[1,10,11].

Since the rate and frequency of transdifferentiation from $3\frac{1}{2}$-day embryo NR is 2–3 times higher than from 8-day embryo NR, we tested the hypothesis that the levels of crystallin mRNA expressed in the earlier stages will be higher than in the later stages.

The hybridisation data (Fig. 1) show that $3\frac{1}{2}$-day embryo eye cups (future NR and PE) express crystallin mRNAs at about 10 times the level found in 8-day embryo NR, while none can be detected in the retina of 1-day-old chicks, by which stage transdifferentiation into lens is no longer possible (ref. 8 and Table 1). Paradoxically, although low levels of crystallin protein are detectable in 8-day embryo NR[12], we have not detected crystallins in $3\frac{1}{2}$-day eye cups, suggesting possible ontogenic changes in the regulation or the translatability of the mRNA.

Evidence pointing to post-transcriptional sequestration for some β-crystallin mRNA has been reported for day-old chick lens[13]. It remains to be tested whether sequestration of crystallin

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Table 1 Transdifferentiation into lens cells from chick embryo neural retina

Age of embryo (days):		$3\frac{1}{2}$	6	8	12	15	17	19	(1-day-old chick)
Time for lentoid formation, days		8–15*	23†	19–25†	25†	28–35†	50†	—†	—†
% of lentoid clones formed		20*		8*	Few*	Few*	—*	—*	
Multipotential clones		+*	Few*	Rare*					
σ-crystallin in lentoids, %		80*	50†	25†	6†	3†	0†	—†	—†
σ : α crystallin ratio		20 : 1*	7 : 1†	3 : 1†	2 : 1†	2 : 1†	α, β only†	—†	—†
Crystallin mRNA compared to day old lens		0.18%‖		0.025%‡					ND‖
Final level of crystallin mRNA obtained in terminal culture compared to 1-day-old lens		17%§		14%‡					
Time required		3 weeks§		6 weeks‡					
Crystallin, as percentage of total soluble protein¶	α	ND‖		0.015%‖					0.04%‖
	β	ND‖		0.04%‖					0.13%‖
	σ	ND‖		ND‖					ND‖

ND, not detectable (<0.01% for all cases except δ-crystallin, <0.005%).
* From refs 3, 6, 7.
† From ref. 8.
‡ From refs 9–11.
§ From refs 13, 14.
‖ This work.
¶ Haemagglutination inhibition assay. Limits of detection for antisera used: α, 0.01%; β, 0.01%; σ 0.005%.

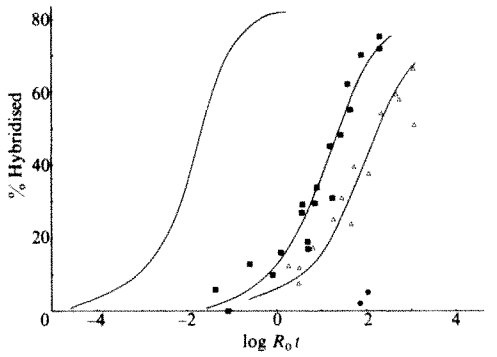


Fig. 1 Lenses from 500 $3\frac{1}{2}$-day-old chick embryos were removed, checked for their integrity and discarded to eliminate all possibility of contamination of eye cups by fragments of lens. The eye cups were then excised and stored in liquid nitrogen. Cytoplasmic mRNA and soluble protein were prepared from two separate such aliquots[10–12]. Neural retina from 100 8-day embryos and retina from 50 day-old hatched chicks was similarly processed. The protein fraction from each sample was analysed by haemagglutination inhibition assay using antisera to crystallins[12]. Total cytoplasmic RNA was prepared from each of these tissues by first homogenising in 0.35M sucrose, 10 mM Tris *p*H 7.5, 50 mM KCl, 1.5 mM $MgCl_2$, followed by centrifugation at 10,000*g* for 10 min at 4 °C. RNA was extracted by addition of an equal volume of phenol/chloroform (1 : 1 by volume), 0.05% 8-hydroxyquinoline, and collected by ethanol precipitation. Poly(A)-containing RNA was fractionated by two cycles of oligo(dT)-cellulose chromatography, and collected by ethanol precipitation as before. Complementary DNA (cDNA) was synthesised from 1-day-old lens[9]. mRNA at 100 μg ml^{-1} was added to a 100 μl incubation mix containing 10 μg ml^{-1} oligo(dT)$_{12-18}$, 75 μg ml^{-1} actinomycin D, 375 μg ml^{-1} bovine serum albumin, 50 mM Tris-HCl *p*H 8.2, 5 mM Mg acetate, 50 mM NaCl, 2 mM dithiothreitol, dATP, dGTP, and dTTP (each at 400 μM) and 45 μM ^{3}H-dCTP (20 Ci mmol^{-1}). Abundant cDNA (complementary only to the crystallin mRNA) was prepared by two cycles of hybridisation to lens mRNA to a R_0t value allowing only hybridisation of the most abundant sequences[9]. Appropriate volumes of mRNA and cDNA solutions in sterile distilled water were mixed, lyophilised and redissolved in 0.5 M NaCl, 25 mM HEPES *p*H 6.8, 0.5 mM EDTA, and 50% (v/v) deionised formamide. Aliquots were heated to 60 °C for 5 min, and allowed to hybridise at 43 °C for various times. The proportion of cDNA in the hybrid was determined by resistance to treatment with S_1 nuclease[9]. ■, $3\frac{1}{2}$-day embryo eye cup mRNA; ●, 1-day-old chick retina mRNA; △, 8-day embryo neural retina mRNA. For comparison, the solid line to the left shows values for 1-day-old lens mRNA (ref. 9).

mRNAs or instability of the crystallin proteins characterise the $3\frac{1}{2}$-day eye cup.

The presence of low levels of crystallins in the freshly excised retina of day old chicks in the absence of detectable crystallin mRNA suggests that those crystallins which are synthesised during the embryonic period are not broken down.

Measurement of hybridisable crystallin mRNA in transdifferentiating $3\frac{1}{2}$-day embryo NR cultures shows that the levels attained in terminally differentiated cultures are similar to those achieved by 8-day embryo NR but these levels are reached by 3 weeks from the $3\frac{1}{2}$-day embryo[13,14] as compared to 6 weeks for 8-day embryo NR. These data, and the data presented here, suggest that mRNA and transdifferentiation potential are related, but we have not yet determined whether the change affects the numbers of cells expressing crystallin mRNA or the levels per cell. This is currently under investigation. Previous data have, however, indicated that some of the cellular conditions and manipulations favouring transdifferentiation exclude cell selection as a possible mechanism[1,4]. Many mRNAs normally coexist in cells, so it seems possible that the ease of differentiation along a particular pathway has a molecular basis in the relative levels of the mRNA appropriate to the end point in that pathway.

If low levels of crystallin mRNA are the preconditions for transdifferentiation into lens, (as we propose here), then cell dissociation followed by reassociation are the permissive conditions, and the directive conditions include cell culture medium, cell density, embryonic age and genotype, which all affect both the direction of transdifferentiation and the relative balance of the different crystallins ultimately synthesised. Evidence for these latter propositions is assembled and discussed more fully elsewhere[1].

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A double-stranded β-helix with antiparallel chains in a crystalline oligo-L–D-peptide

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There is much interest in the ionophore-mediated and channel transmembrane transport of alkali metal ions. Valinomycin and gramicidin A, which is a linear pentadecapeptide with alternating L–D-residues, are classic representatives of carrier and channel-type ionophores, respectively, and their structure–function relationships are being intensively studied[1–4]. Helical structures for alternating D,L peptides have been proposed from conformational energy calculations[5–7], and from experimental spectroscopic data single-helical structures have been postulated for gramicidin A (ref. 8). Stereochemical and energy considerations[7,9] have suggested that polypeptides with alternating L- and D-amino acid residues may take up coaxial double-helical β-structures, both parallel and antiparallel, which are stabilised by systematic interchain NH....OC hydrogen bonds. Experimental results supporting the occurrence of structures of this kind have been obtained by Veatch, Fossel and Blout[2] for gramicidin A in nonpolar solvents, and by Lotz, Colonna-Cesari, Heitz and Spach[10] for poly-(γ-benzyl-D,L-glutamate) in the solid state. However, there has previously been no experimental determination of the detailed structural and conformational parameters characterising these structures. We now provide detailed conformational parameters of a double-stranded β-helix with antiparallel chains obtained by single crystal X-ray analysis of the model L–D peptide Boc-(L-Val-D-Val)$_4$-OMe (ref. 11).

The compound investigated crystallises from ethyl acetate solutions in the trigonal $P3_221$ space group with cell dimensions $a = 12.760$ Å, $c = 63.190$ Å and $d_{calc.} = 1.05$ g cm^{-3}, based on monohydrated units ($C_{46}H_{84}N_8O_{11}.H_2O$) and $Z = 6$. Data, collected on the Enraf–Nonius CAD-4 PDP 11/34 diffractometric system of the Centro di Metodologie Chimico Fisiche at the University of Naples, consisted of 6,399 measured reflections, 2,721 of which had a net intensity $>3.0\sigma$ (I). The structure was solved using Multan and has been refined by a least squares procedure, with isotropic thermal parameters for all atoms to an R factor of 0.13 for the observed reflections.

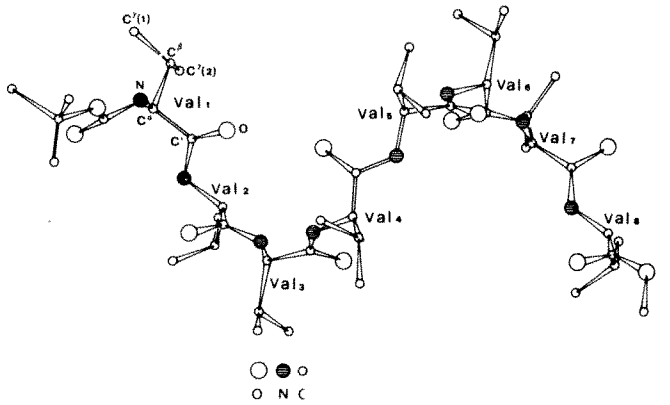


Fig. 1 Molecular model of Boc-(L-Val-D-Val)$_4$-OMe as viewed along the b axis. The eight valine residues are schematically indicated; only the first is labelled.

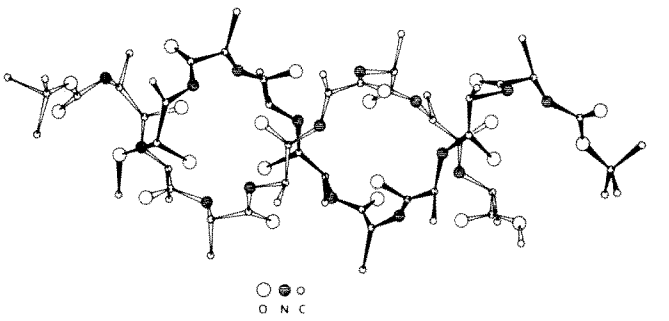


Fig. 2 The double-helical dimer as viewed along the b axis. The side chains are omitted from the model for clarity.

Figure 1 shows the molecular model of the octapeptide chain. Its structure consists of dimers (Fig. 2) in which each peptide chain is related to the other by a crystallographic twofold axis perpendicular to the helix axis, giving rise to a double-stranded, left-handed helix of antiparallel chains. Seven independent (14 in one dimer) NH....OC intermolecular hydrogen bonds link two antiparallel chains, with N....O distances of 2.7–3.0 Å. Only one potential hydrogen bond donor per chain is not involved in hydrogen bonding. The conformation of each chain can be described by the φ and ψ conformational parameters. They assume values in the range of $-148° \leq \varphi \leq -112°$ and $98° \leq \psi \leq 122°$ for the L-residues and $80° \leq \varphi \leq 129°$ and $-163° \leq \psi \leq -152°$ for the D-residues. Side chains assume different conformations for L- and D-residues; all four D-residues have the two methyl groups of the isopropyl moiety in g_+ and g_- conformations (close to $+60°$ and $-60°$) with respect to the N atom, whereas in L-residues the g_+, t conformation ($+60°$, $180°$) is observed three times and the g_-, t conformation ($-60°$, $180°$) only once. Along the axis of each dimer four distinct sections (about 4 Å apart) can be seen, in each of which four carbonyl oxygen atoms lying in a non-planar arrangement, could be available for possible coordination to metal ions within the core of the chain. The distance of each carbonyl oxygen from the chain axis is 2.5–2.9 Å. Projections along the dimer axis clearly show the steric hindrance produced by the N- and C-terminal blocking groups which partially close the mouth of the 'cylinder'. The water molecule is hydrogen bonded to the urethane carbonyl oxygen and a symmetry-related water molecule. All peptide linkages, as well as the urethane bond, occur in the *trans* configuration ($\omega = 180$), with maximum $|\Delta\omega| \leq 6$.

The dimer can thus be regarded as a cylinder with an hydrophilic inner core of almost planar peptide bonds nearly parallel to the chain axis, and an hydrophobic outer layer of isopropyl groups of the valyl residues. The length (from N_1 to C_8') and average inner diameter of the cylinder are 14.8 Å and 5.1 Å, respectively.

The double-helical structure of the dimer described here can be considered a stereochemical model of the antiparallel β-double helix proposed by Blout and coworkers[3] for species 3 of gramicidin A.

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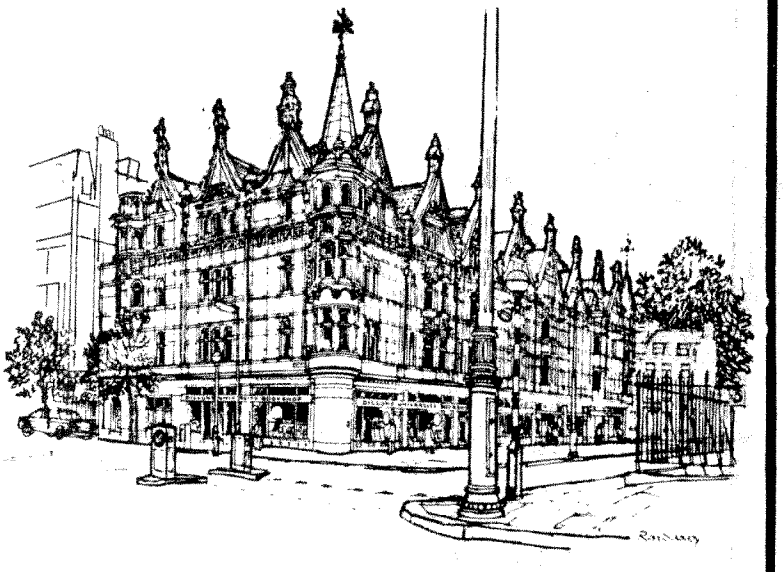
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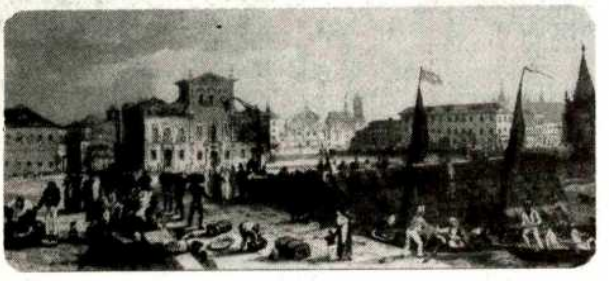
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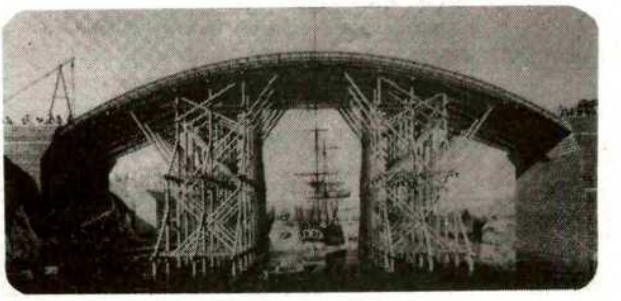
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CHRISTMAS BOOKS

The guiding star

W. H. McCrea

The Star of Bethlehem Mystery. By David Hughes. Pp. 218. (Dent: London, 1979.) £7.95.

THE story of the star of Bethlehem comes to us in the vivid words of Matthew 2: 1-12, and nowhere else in the *Bible*. The mystery in the title is that of the star's identity. If a disproportionate part of this review is taken by the reviewer's response to the challenge of David Hughes' interpretation, it is in tribute to the fascination of the book. That interpretation is as follows:

The year 7 BC saw the rare occurrence of a triple conjunction of the planets Jupiter and Saturn. As its most relevant consequence, it caused these planets to keep about one degree apart in the sky from mid-September to early December. The planet-pair during that interval was the 'star' of Bethlehem. In extant cuneiform texts, Babylonian astronomers had accurately predicted its observable behaviour. The Magi of the story were Zoroastrian priests schooled in such astronomy. At the time they shared a widespread expectation of the manifestation of a Messiah. Their sophisticated astrology made them take some — predicted or observed — feature of the behaviour to augur this Messiah's birth and to denote that it would occur amongst the Jewish people in the land of Palestine. Such was their conviction that a group set out to discover the child. They made for Jerusalem as the chief city of Palestine; there they created a stir by asking about a new-born 'king'. In consequence, King Herod directed them to Bethlehem. The 'star' reappeared, and somehow indicated their goal. There the Magi paid joyful tribute, and offered gifts which they had dedicated to this consummation.

Ruthlessly distilled, such is the account emerging from Hughes' comprehensive presentation of the astronomical and historical evidence. His astronomical conclusions are generally wholly convincing. However, there are difficulties in the account as a whole. First, as we shall see, it explains too much. Then, although Hughes has convinced me that he could have convinced the Magi that their own conventions would produce the suggested astrological prediction, there is no known evidence that the Magi made this prediction. Again, by inadvertently implying such a success for astrology, the account apparently resolves one mystery by creating a worse mystery.

Let us consider an alternative approach, and let us do so with reference to the version of the Matthew story in the *New English Bible*. The quoted passage is succinct; let us be the same. We proceed on the basis — to be tested — that it is historical. Matthew could not know the story from personal experience. He introduces the astrologers (as the Magi become in *NEB*) simply as "arriving" in Jerusalem. There is no suggestion that a star induced them to come, none that it guided them. So they came as ordinary visitors. We know there were periodical visits by "inhabitants of Mesopotamia" (Acts 2:9) and the like; we infer that the astrologers were amongst such visitors. But this particular visit turned out to be uniquely eventful; the homecoming participants would rehearse the tale until it became a set piece. Other visitors would carry it back to Jerusalem, and Matthew has passed it on to us. In confirmation, linguists remark how each mention of the star is in terms that astronomer/astrologists would use. Also, the passage is singular in that Matthew finds no prophecy to quote about the star. If the account is historical, we are thus most probably dealing with the astrologers' version.

When the account opens, it all but explicitly admits that the astrologers then knew about the birth of Jesus as something out of the ordinary. So they would cast about for a portent. An observed acronychal rising of a 'star' supplied it. Portents belonged to rulers, so the child was 'born to be king' — of the Jews, for he was in Judea. In spreading this notion, we cannot know how much the astrologers themselves believed. Anyhow it upset King Herod, who would tolerate no threat to his dynastic obsession. He asked his advisers about the birthplace of "the Messiah" — presumably trying to conceal his motive by not saying "king". Since Matthew has them replying in the identical words in which he had just recorded the birth of Jesus, he appears to tell us that they had knowledge of the birth; but they covered up by "referring" Herod to a prophecy about Bethlehem. He surreptitiously met the astrologers, interrogated them about the star, despatched them to Bethlehem, and ordered them to report back.

In Bethlehem, the astrologers found the "place where the child lay", and soon they "entered the house". In the intervening moments "they were overjoyed at the sight of the star . . . which they had seen at its rising". It was early evening because, although the star was visible, the hour was suitable for the strangers to visit the child and his mother. So the star had just "come out", and it could not have guided them on the way. Matthew does not say it did, but simply that it was there ahead of them. Granting that it was a real star, the sight would have been the same that evening anywhere else in Palestine, and much the same any other evening within days. But to astrologers a star would be of interest only if doing something special — like rising acronychally; or seen at a special moment — like this, when their pretensions were on trial. The astonishing sight at this moment of the self-same star that they had adopted as portent seemed miraculous confirmation of their claims, no matter how little faith they had in them — hence their rapture. So they entered, "saw the child with Mary his mother" and saluted him. The record says, "then they opened their treasures"; that they had to do this, between the salutation and offering gifts, implies that the turn of events had taken them by surprise. Then they went home; and that is the end of what we may take to be their own story.

Matthew's continuation shows that the astrologers evidently told Joseph of their intention to evade Herod. The simplest conclusion for Herod would have been that they had failed in their mission. His murderous reaction indicates that some rumour of their finding the child got round to him.

This narrative seems to overcome the difficulties found in Hughes'. It purports to account for nothing beyond what Matthew tells us. Although it takes account of the behaviour of astrologers as such, nothing depends upon any interpretation on their part beyond what is directly implied by the text as we have it, and nothing implies any success or benefit attributable to astrological prediction. The climax of Matthew's account of the astrologers in the slaughter of the Innocents is indeed a terrible indictment of their activities, as well as of Herod.

Certain possible difficulties mentioned by Hughes himself are also resolved. For instance, the fact that two and only two sightings of the star play any part is automatically explained. Also, as the first sighting is here held to have had nothing to do with the astrologers setting out for Jerusalem, it could have occurred any time up to their arrival; so it denotes no constraint upon the duration of their travels.

On the revised account, Babylonian astrological conventions carry less weight in attempts to identify the star. Nevertheless, purely astronomical

considerations, and elementary regard for what could interest astrologers, still seem almost overwhelmingly to support the identification with the planet-pair Jupiter and Saturn favoured by Hughes. Or it might be that 'the' star was just Jupiter, with the attendant Saturn making it more than usually noticeable or interesting for the time being.

The fact that the account can be so readily related to a known astronomical phenomenon, and its remarkable internal consistency, are strong evidence for its historicity. If both lines of evidence are accepted, they indicate that the Magi/astrologers went to Bethlehem in 7 BC sometime after mid-September, as Hughes would agree, but some of his other time-constraints may be relaxed somewhat.

It was David Hughes himself who, by his article in *Nature* (**264**, 513-517; 1976) on this most famous star of all, initiated the recent renewed interest of, particularly British, astronomers in the subject. In his book, he now surveys it in the widest possible setting. He reviews the results of an enormous amount of published scholarship in biblical and historical studies, in archaeology and anthropology of the Middle East, and in astronomy itself. Naturally, he takes full account of the discussions instigated by his *Nature* article. He has devoted acute critical and constructive thought to collating this vast material, with most illuminating results. He re-discusses probably every serious suggestion that has ever been advanced to account for the 'star' by astronomical phenomena or other natural processes. He writes with vigour and clarity, reinforced by numerous well-conceived tables and diagrams, happily-chosen plates, and a full bibliography. The wealth of background and sequential material is bound to make it harder to trace the central theme of the identity of the star and what it can tell about the birth of Jesus. However, most readers will be fascinated by it, as well as by this theme itself.

The planetary conjunction hypothesis regarding the star goes back at at least as far as Kepler. A leading modern exponent, particularly in regard to its setting in Babylonian astronomy is K. Ferrari d'Occhieppo, to whose valuable monograph *Der Stern der Weisen* (Vienna, 1968, second edition 1977) Hughes makes frequent reference. Hughes' book is the only full account in English, and I think it is the most comprehensive work by any astronomer on the subject of the 'star'.

The book inspires yet another line of thought. Questions of significance will be discussed for evermore. But, wherever possible, matters of simple fact should be settled once and for all. In regard to an important date, for example, it ought now to be possible to use a computer to sift all the evidence, test it for compatibility and so forth, and in at least some cases produce a uniquely acceptable solution. In other cases a computer might determine the date by predicting testable consequences of all reasonable hypotheses about it. The possibilities seem unbounded. □

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Biological analogy

Martin Pollock

The Evolution of Designs: Biological Analogy in Architecture and the Applied Arts. By P. Steadman. Pp. 276. (Cambridge University Press: Cambridge and New York, 1979.) £9.50.

THIS book is essentially an analytical description of the evolution of design in the construction of human artefacts, being focused, as the subtitle indicates, on architecture and the significance of biological analogy in attempts to understand the principles involved.

The biological analogy is, indeed, a fertile and stimulating one and there is plenty of interest in how Professor Steadman pursues, criticises and finally rejects it as "false". Close comparisons between living organisms and man-made structures can be drawn at many different levels: the whole system designed "for a purpose"; classification of different but related types; structural anatomy (endo- and exoskeletons in both cases); ecology (form related to function and the environment); evolution (Darwinian versus Lamarckian); ornamentation (vestigial organs in plants and animals and so-called "skeuomorphs" in architecture that involve conversion of originally necessary features into purely decorative patterns); and many others, all considered in some detail.

During the course of this comparison, the author discusses various attempts to develop a general understanding of the principles of design over the past 150 years. Amongst these, particular critical attention has been paid to the general evolutionary doctrines of Herbert Spencer, to the fascinating "Purism" of Le Corbusier and Ozenfant, whose views on the basic banal forms they called "objets-types" (such as the bottle, tobacco-pipe, drinking glass, and so on) which, having completed their evolution to combine maximum utility with maximum manufacturing economy and acceptability (including aesthetic appreciation), correspond closely to Darwinian principles applied to artefacts; and, in more recent times, to Christopher Alexander whose views on the "unselfconsciousness" of the better designs in architecture are presented as being close to the biological analogy; and to David Clarke's *Analytical Architecture* (1968), which also argues against cultural systems having a "life of their own", considering them rather as part of biology, comprising "information systems of cumulatively acquired knowledge, partly replacing instinctive behaviour in man and selectively advantageous in his struggle for survival" (page 220).

All this is of great interest and value. But the style is rather heavy and the presentation somewhat confused — perhaps even misleading at times — largely because of this concentration on the significance, or otherwise, of a biological analogy. Indeed, the concept itself of *analogy* (defined in the *Oxford English Dictionary* as "resemblance of form or function without identity of essence") differs according to taste and habit. Sometimes it is simply a question of similarity without identity: for instance, most buildings, like many organisms, need light and insulation and internal structural support; they are inevitably subject to the same chemical, physical and mechanical forces of their environment and must therefore have features in common. A very different type of analogy is primarily anthropomorphic — sometimes to the point of being poetic fancy, such as the resemblance of the façade of a house to a human face. After all, man is always constructing models of what he perceives; and because he finds it hard to have totally new ideas, he is always making comparisons and extensions, usually with reference to human experiences already encountered. He will see himself and familiar objects in almost anything. To pay too much attention to analogy (in all its varied forms) in order either to discard or accept any particular interpretation can therefore be misleading. Its value, I would argue, is in helping to visualise alternative ways of approaching an understanding of a problem.

Philip Steadman rejects the "biological analogy" largely because the evolution of design, like other forms of cultural expression, is Lamarckian (that is, directly moulded by the environment) rather than Darwinian (due to selection of a very few types out of a huge number of possible variants) which is the currently accepted theory of organic evolution. Even here he admits that the situation is not perhaps as clear cut as might at first appear: successful designs *do* survive through some sort of selection, from a large variety, by human choice which acts as the controlling environment as well as the apparently direct source of the various designs. But most biologists would agree that cultural evolution, whatever its mechanism, does not involve the permanent stable change in DNA composition that occurs in the evolution of living organisms themselves. And Popper is quoted (page 216) as pointing out that if all literature and other types of artefact were totally destroyed, the

human race would have to start again almost from scratch. Only memory would remain and that would persist through generations only in so far as verbal transfer of information from one person to another continued. But we really know very little about human evolution over the last 50,000 years — that is, during the period when man himself has been modifying his own environment and so possibly directly affecting the manner in which the environment has been playing a part in his evolution, at least to an extent far greater than have other organisms.

This only emphasises another problem to be faced in the exploitation of biological analogy; namely the importance (and difficulty) of getting the biological situation right in the first place.

A simple example of this would, indeed, be Steadman's second argument for rejecting the Darwinian analogy (page 101) which is that cultural evolution is "reticulate"; that is, it combines elements from different origins and is therefore fundamentally distinct from the Darwinian "tree" in which the branches always diverge and never rejoin. Well-versed in biology generally, the author is not here up-to-date, as possible instances of "reticulate" evolution in biology are becoming more and more convincing and the prokaryotic origin of both the chloroplasts and mitochondria of eukaryotes is now generally accepted: a rather pretty analogy with (for instance) the common practice in early Islamic architecture of actually using (not just copying) pilfered Roman pillars for decorative support of vaulting such as that of the famous Omayyad mosque in Damascus.

But this is really neither here nor there in the study of the evolution of design generally. Reference to analogy is useful for hanging a discussion upon but, in the last resort, the relevant processes have to be analysed in terms of the direct influences that operate. And the problem centres on the extent to which man's activities can be considered to be simply one aspect of the way some organisms operate: strictly a problem in biology, complicated though that may be. The opposite approach, favoured by Professor Steadman, is that man's cultural expression must be studied, at least some extent, in its own right: there is such a thing as design *per se*; it is the result of conscious mental activity and cannot be reduced by scientific dissection to analysis in strictly biological terms. And from that angle one is plunged (if not explicitly) into all the deep conundra that have plagued philosophers from time immemorial: determinism, intrinsic free-will, reductionism, dualism, and so on.

At the end, the author asks the question: "What are the features or properties of artefacts of all kinds to which scientific study should be directed and about which scientific prediction might be made?" (page 238). To the question: "Can design be made scientific?", he answers that at least *some* features are beyond scientific attack. A closely related and even more critical question might be: "To what extent do we need to study the subjective thoughts and feelings of human beings — as distinct from what they *do* (which is how we study other organisms) — in order to understand the history of processes such as the evolution of design?". And if the former seems important we may further ask: "How do we do it?" and then: "Can we do it scientifically?". □

Martin Pollock is Emeritus Professor of Biology, in the University of Edinburgh, UK.

New English *Origin*

Richard Dawkins

The Illustrated Origin of Species: An Abridged Edition of the Sixth Edition. Abridged and introduced by Richard E. Leakey. Pp.240. (Faber and Faber: London, 1979.) £8.95.

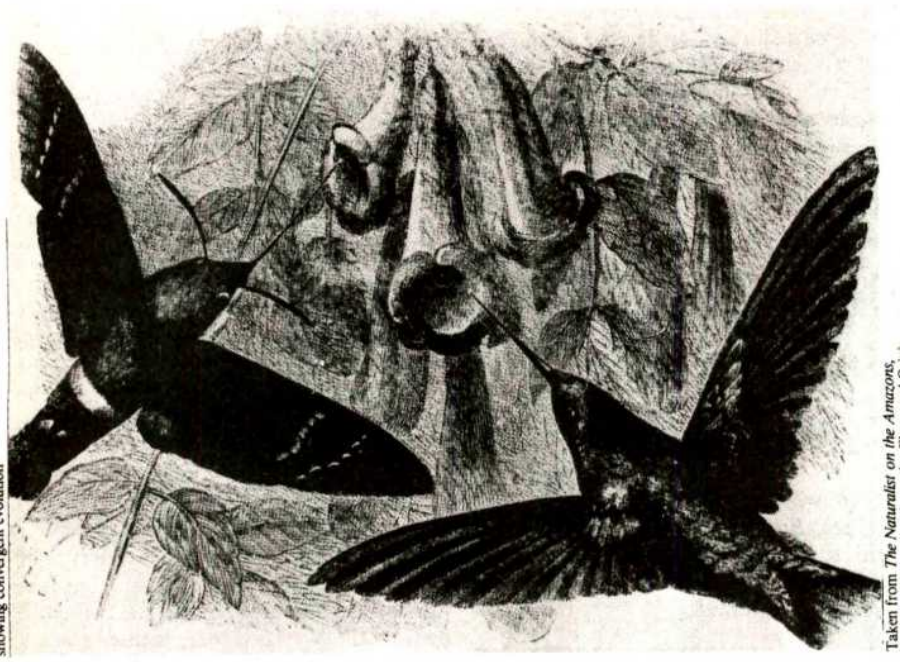
An invitation to review the *Origin of Species* in *Nature* is too much for the humble modern biologist to pass up. What line to take? "Would be better if he had stuck to pigeon breeding?" No, that's been had before. "Sexist, and unconsciously favours the status quo?" Better, but those in a position to see the funny side haven't the sense of humour to do so. "Cannot be saying anything very profound, since any layman can understand him?" Ah, but that does not mean that any *fool* can understand him. On the contrary he has been comprehensively, comically, misunderstood by many who were not laymen and not fools. The patient effort Darwin put into advocating his thesis, the persuasive, cogent clarity that he achieved, lead one to the salutary realisation that ideas do not have to be formidably complex, smothered in mathematical barbed wire, in order to be utterly incomprehensible to unprepared minds. They only have to be revolutionary. But this reviewer is getting above himself. It is his humbler task to review a pleasing picture book for the Christmas coffee-table market.

In a sense, everything is in the idea. Once you have *thought* of producing an illustrated *Origin of Species*, the rest is money for old rope. Well, and scissors and paste and a lot of hard work phoning round to the photographic agencies. I would certainly like to salute the unnamed employee (or ex-employee) of the Rainbird Publishing Group who thought of it. If ever an illustrated book conjured up vivid pictures in the mind of the reader, it is the *Origin of Species*. Darwin wrote before colour photography was invented, and he included only one technical diagram in his book. But he was such a passionate advocate, such a tireless seeker after honest ways to make his meaning plain to everyone, that I cannot doubt that he would have welcomed all the visual aid that modern photography can offer.

The idea, then, is an excellent one, and I find nothing irreverent or reprehensible about putting it into practice, except insofar as it may pre-empt later bites at the same cherry. It is, evidently, an idea whose time has come, a hot property in the publishing world. Only about a year ago I recall a colleague, who had been invited by a publishing company to edit a similar illustrated *Origin*, discussing how he might do it. An artist as well as a scientist, he saw the task as a labour of love for Darwin, and he would have used only contemporary Victorian illustrations. I (who coincidentally and simultaneously had been invited to edit an illustrated *Origin of Species* by yet another publisher!) was more of the Leakey school of thought. If Darwin had wanted to illustrate his book with Victorian prints, I suggested, he could have done so himself. I would have used modern pictures, as technically perfect as I could find. I think Darwin would have appreciated that labour of love more, and I am glad that Leakey and the Rainbird team elected to do it that way. My colleague and I, incidentally, both declined our invitations, but I still have the notes I made as I reread the *Origin* with illustrations seriously in mind, and it was consequently of absorbing interest to me to see how Leakey and Rainbird had actually done it.

I think they have done it rather well. In addition to excellent black and white and colour photographs, they have used artistic drawings and paintings, clear maps and diagrams, and even a few Victorian prints. The captions are well done, frequently beginning with a relevant quotation from Darwin's text and ending with an up to date comment from Leakey. Someone who simply browsed through the pictures would come away with a good feeling for the diversity and complexity of life, with a sense of something needing to be explained which might inspire him to read the text.

I am less happy with the fact that Leakey has comprehensively bowdlerised the text into a kind of *Reader's Digest* version. No doubt some cuts were necessary; I am sure Leakey has succeeded in his stated aim of eliminating Darwin's "tendency to Victorian wordiness", and that he can be trusted to "in no way change Darwin's original meaning," but even so! This may be the only copy of the *Origin* that many people possess, and they will want to be able to refer to, or quote, exactly what the man said. It might be thought that the problem could have been solved by indicating omitted passages by dots, and



Humming-bird hawk-moth and humming bird, showing convergent evolution

Taken from *The Naturalist on the Amazons*, by Henry Bates, and from the *Illustrated Origin*

this would have been so if Leakey had contented himself with cutting out definite large chunks or whole chapters. But in fact he has done such a thorough (and actually rather good) editing job that scarcely a single sentence is exactly as Darwin wrote it, and the pages would have become a sea of dots. Leakey insists in his Introduction that, in making these cuts, he has not "tried to make Darwin more modern than he was, since he can stand on his own feet." But consider the very first whole sentence omitted: "There is, also, some probability in the view propounded by Andrew Knight, that this variability may be partly connected with excess of food." Darwin was wrong here, and the omission of the sentence (with no indication that anything has been cut) cannot fail to make Darwin seem more modern than he was. Darwin can stand on his own feet, certainly, and he doesn't need that kind of help.

Turning to the rest of the Introduction, I sympathise. The *Origin of Species* must be one of the most introduced books in science, and the supply of original things to say must be running low. In the circumstances Leakey does what I would have done, and contents himself with a smooth and unexceptionable record-sleeve job, a little standard history and a little modern biology, especially genetics, bringing us up to date on what has happened since Darwin's time. For me, as, I think, for Leakey, reflection on such matters has the odd effect of underlining Darwin's own remarkable prescience. He was, in Michael Ghiselin's words, "a genius of the first rank and a man of immense learning and he often worked a century or more ahead of his time." Natural selection theory is in an exciting and fast-moving phase just at the moment, but in many respects the 'revolutionary new' insights of the 1960s and '70s represent a rediscovery of something that had been lost — Darwin's own uncompromising grasp of the ruthless individual struggle in the economy of nature.

For this reason I am slightly sorry that Leakey chose the sixth edition as the basis for his abridgement. The first edition, though not Darwin's parting shot on the subject, is of greater interest, both historic and scientific. It is of greater historic interest as the version that actually hit the Victorian solar plexus. But, paradoxically (this, too, is not an original point!), it seems scientifically more advanced than the sixth edition. The sixth edition includes Darwin's attempts to rebut contemporary objections to his thesis. These objections now have a fusty, dated air, and Darwin's replies to them inherit the same old-fashioned feel, something that the first edition, in its freshness, does not have. As is well known, Darwin's attempts to grapple with objections such as those of the great physicist Lord Kelvin, led him up the Lamarckian garden path much further in the sixth edition than in the first. Kelvin's calculation suggested that the Earth simply was not old enough to allow the evolutionary process that Darwin postulated. Darwin, humble non-mathematician that he was, had to accept Kelvin's authority and this led him into erratic special pleading. As Eisely has remarked, "today there is a tendency in some quarters to regard the physical sciences as superior in reliability to those in which precise mathematical adeptness has not been achieved. Without wishing to challenge this point of view, it may still be worth a chastening thought that, in this long controversy extending well over half a century, the physicists made extended use of mathematical techniques and still were hopelessly and, it must be added, arrogantly wrong". Darwin had been right all along, in the first edition, and the objections to his theory that he anticipated and refuted in the first edition have proved the durable ones that we still meet. Leakey actually seems to have an impoverished view of the first edition: he says that Darwin, faced with the difficulties of natural selection, "began to search for additional mechanisms. Darwin wrote another book about one of these accessory mechanisms — sexual selection — and he discussed it briefly in the sixth edition of *The Origin of Species*." This seems, wrongly, to imply that the first edition lacks the section on sexual selection.

The illustrations in this book are well chosen and attractively presented. Even if they were not, it would be hard for the formula to fail. Open the *Origin of Species* anywhere, and you immediately feel you have walked into the presence of a timeless master, whose location in the Victorian era would be hard to guess but for his ignorance of Mendelism and his odd deployment of commas (which, now that I think about it, Leakey also seems to have taken it upon himself to correct). To complete my quotation from Ghiselin, "What a pleasure it is to bask in the sunlight of his intellect". The pictures don't spoil my pleasure, and if they help to spread some sunlight over the coffee-tables of Christmas I am all for them. □

Richard Dawkins is Lecturer in Zoology at the University of Oxford, and a Fellow of New College, Oxford, UK.

Highly popular science

THERE will be little in *Scientists at Work: The Creative Process of Scientific Research* (edited by John Noble Wilford; Dodd, Mead: New York; $9.95), a collection of articles by journalists from the *New York Times*, that will be recognisable to working scientists. The articles are short, superficial, uncomfortably chatty and devoid of any critical approach to their subject. The writing is of the "Chilly winds howled across a massive stone overhang where a band of Stone Age hunters huddled around a campfire" type. The actual steps of the tedious construction of the elements of Stone Age life are omitted in favour of flowery and simplistic imagery. And it is the same throughout this collection of the "highly popular" *Scientists at Work* series which "introduces a world rarely seen or imagined by non-scientists".

Why is science reporting so bad? The editor informs us that none of the nine authors have less than ten years reporting experience. In addition we find that no effort or expense was spared to get the stories. "Interviews . . . extended over a period of several days" and the work involved "staying up all night at Kitt Peak

Studies in Archaeological Science

Principles of Archaeological Stratigraphy

Edward C. Harris

December 1979/January 1980, xii+136pp., £8.00/ $18.00 0.12.326650.5

This book is not only the first to set down the basic principles of archaeological stratigraphy; it also contains many new ideas on the subject. In particular, the use of the Harris Matrix, a method for analysing and recording archaeological stratification which was invented by the author in 1973. The book has three main sections. The first examines the historical development of some aspects of geological and archaeological ideas on the theory of stratigraphy, including the excavation and recording of stratification on archaeological sites. The second part considers the individual characteristics of archaeological stratification and methods by which it is recorded and its post-excavation analysis. The final part of the book is a series of appendices outlining modern recording procedures, a brief discussion of the development of the Harris Matrix and a glossary of terms used in archaeological stratigraphy.

Biological Techniques Series

Time-Lapse Cinemicroscopy

P. N. Riddle

October 1979, xiv+122pp., £8.50/ $18.00 0.12.588060.X

The realization of the analytical powers of time-lapse cinemicroscopy, with the increasing emphasis on quantitation, is a relatively new development. Dr. Riddle's work in organizing a highly efficient cinemicroscopy service for cancer research places him exactly in an authoritative position to present this account of apparatus, techniques and their applications. Dr. Riddle's approach is unusually valuable because he looks in detail at a range of instrument types, explaining their interdependencies and linking this to an exploration of how standard techniques may be included in cinemicroscopy. His inclusion of many references indicating where specialized uses may be followed up is most useful. Constructive discussion throughout the text is complemented by frequent references to potential problems and their possible remedies.

The Institute of Mathematics and its Applications Conference Series

The Mathematics of Hydrology and Water Resources

edited by E. H. Lloyd, T. O'Donnell and J. C. Wilkinson

October 1979, xii+138pp., £8.80/ $18.50 0.12.453350.7

Hydrology and water resource engineering are areas which have until very recently enjoyed a lesser degree of support from professional mathematicians than have many of the more traditional engineering disciplines. This conference was arranged with the advertized aim of encouraging communication and interaction between hydrologists and mathematicians, and improving and extending the application of mathematical techniques to problems of hydrology together with the neighbouring fields of civil engineering, meteorology etc.; and mathematics, together with probability and statistics. The main aim of the work is to combine complementary approaches by applied mathematicians and practising engineers for the assistance and edification of water engineers and those carrying out research and advanced studies in hydrology and applied mathematics.

Antibiotic Interactions

edited by J. D. Williams

December 1979/January 1980, xii+184pp., £10.00/ $23.00 0.12.756350.4

The publication of these proceedings marks the first of an occasional series of meetings concerning various aspects of infection which are being held under the sponsorship of Beecham Research Laboratories and are entitled The Beecham Colloquia. The series aims to create the opportunity for multi-disciplinary discussions into various key facets of the control of infection. This first meeting was held at the Medical Society of London and brought together 24 participants from Europe and America. They discussed the development of antibiotic combination therapy and the role of such therapy in the management of infection in man. Ten papers were presented giving up-to-date information and expert opinion on antibiotic interactions and the advantages and disadvantages of combined therapy. A great deal of the subsequent discussion has been included in this book.

Special Publications of the Society for General Microbiology 2

Adhesion of Microorganisms to Surfaces

edited by D. C. Ellwood, J. Melling and P. Rutter

December 1979/January 1980, viii+216pp., £10.00/ $23.00 0.12.236650.6

The problem of bacterial adhesion to surfaces is a rapidly expanding field of interest with great relevance to many diverse areas, from medical and dental research to soil science, from marine fouling to the fermentation industry. This book, which is the second in the series of publications arranged by the Society for General Microbiology, is a timely review of the subject and attempts to introduce a physico-chemical approach to the problem. The contributions are based on papers presented at a joint meeting between the Cell Surfaces and Membranes Group and the Ecology Group of the Society for General Microbiology, and the Microbiology, Fermentation and Enzyme Technology Group of the Society of Chemical Industry. The book starts with two review chapters, the first describing the physical chemistry of adhesion related to small particles, and the second discussing bacteriological aspects of adhesion. The subsequent chapters describe particular situations where bacterial deposition and accumulation is a problem. The final chapter is a provocative discussion of bacterial adhesion to eukaryotic host cells.

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Ontario M3C 2A1, Canada

Neoplastic Transformation in Differentiated Epithelial Cell Systems *in vitro*

edited by L. M. Franks and C. B. Wigley

December 1979/January 1980, xii+312pp., £14.40/ $33.50 0.12.266260.1

This monograph offers the first review, with much unpublished new information, of some of the epithelial transformation systems now in use in the study of carcinogenesis. Originating from an Imperial Cancer Research Fund workshop for scientists working in the field of epithelial carcinogenesis, it presents papers given at that event and other invited contributions. As well as reviewing recently developed systems, some of the technical problems encountered are outlined and possible future developments are discussed. In short, it provides a survey of the present state of our knowledge of this important new branch of the study of carcinogenesis and offers a useful introduction to the field for fugitives from the fibroblast. Cell biologists, biochemists and molecular biologists, and particularly cancer researchers cannot fail to find this stimulating volume of much interest in their work.

Society for Applied Bacteriology Technical Series No. 14

Identification Methods for Microbiologists **Second Edition**

edited by F. A. Skinner and D. W. Lovelock

December 1979/January 1980, xii+312pp., £14.80 $34.50 0.12.647750.7

Since its original publication in 1968 as volumes 1 and 2 of the Society for Applied Bacteriology's Technical Series, *Identification Methods of Microbiologists* has proved beyond doubt to be of most practical value to the scientist when identifying particular groups of microorganisms. Many significant advances have been made, since then, in identification methods for certain genera and there have also been some changes in the taxonomic structure of some genera following the publication of the new Bergey's Manual in 1974. This second edition brings up-to-date in one volume chapters from both parts of the original edition, enabling the practical microbiologist to use the most recently established methods of identification.

Nature and Origin of Carbohydrates in Soils

M. V. Cheshire

December 1979/January 1980, viii+216pp., £15.80/ $36.50 0.12.171250.8

This volume examines the methods which have been devised to overcome the unique problems of isolating, purifying and characterizing carbohydrates in soils. A number of recommended procedures are given in detail and the physical and chemical properties of the isolated polysaccharides, including structure studies, are described. This account of soil carbohydrates is not restricted to their chemistry but includes discussion of the effects of climate, various agricultural practices, the decomposition of microbial and plant remains and the synthesis of new carbohydrate. Although primarily a reference work for soil scientists, agronomists and workers in related subjects, it is hoped that this book will provide a stimulus to further research on this vital area of soil fertility.

European Monographs in Social Psychology No. 18

Towards Understanding Relationships

Robert A. Hinde

December 1979, xii+370pp.
Hardback: £15.80/ $36.50 0.12.349250.5
Paperback: £7.80/ $18.00 0.12.349252.1

A science of interpersonal relationships which comes to terms with the dialectic between personality and relationships on the one hand, and that between relationships and the social environment on the other, could provide a strong link between diverse branches of social science. One section of the book is concerned with an initial requirement for such a science: an adequate descriptive base. Another main section explores a number of areas which may yield principles for understanding how relationships work or fail to work: studies of relationships in the context of a social group; dissonance and balance theories; learning, exchange and interdependence theories; and relationships seen as systems involving positive and negative feedback. Principles from each of these areas are related to the descriptive base. Finally, some developmental aspects are considered.

Constructs of Sociality and Individuality

edited by P. Stringer and D. Bannister

November 1979, xiv+350pp., £17.20/ $41.00 0.12.673750.9

This is the first book in its field to examine systematically the implications of Kelly's personal construct theory for social psychology. The origins of the work can be found in the papers presented at the 2nd International Congress on Personal Construct Theory (1977). Five of these papers — rewritten and expanded — have been included in this volume. The remainder were written especially for this volume with the exception of Kelly's "Social Inheritance" (1930).The book is divided into three sections, covering theoretical and meta-theoretical implications, the value of methods derived from the theory — for example grid method in socio-psychological fields — and finally a number of exemplary research studies. Many contributions to the work focus particularly on applied fields in social psychology covering such areas as politics, education, psychotherapy, relationship formation, social skills, and the law and rule systems.

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Physical System Modelling

P. E. Wellstead

December/January 1979, x+280pp., £18.00/ $41.50 0.12.744380.0

All naturally-occurring physical systems use and process energy; the perceived dynamical behaviour of any system is simply the outward manifestation of this energy transaction. Whilst this notion of energy as a unifying agent is not new, Dr. Wellstead uses it here for the first time as a framework within which to study the various techniques of system modelling. The main part of the text is, therefore, devoted to the established methods of network analysis, variational analysis and bond graph analysis. Some highly specialized areas are excluded to enable the author to introduce a series of case studies that are designed to underline the link between the modelling process and subsequent simulation, design or control studies.

Chemical Thermodynamics

M. L. McGlashan

December 1979/January 1980, xx+345pp., £18.00/ $41.50 0.12.482650.5

This is a book written for chemists by one of the world's leading experts in chemical thermodynamics. Unlike other accounts of the subject, it stresses the fact that thermodynamics is an experiemental field, yet one that is securely founded on exact algebra. Linking these two aspects, Professor McGlashan demonstrates how all the equations of chemical thermodynamics can be deduced from a few axioms and how the physical quantities symbolized in those equations can be measured in the laboratory. Starting out with the aim of showing how and why thermodynamic equations are so useful to chemists, the author discusses those instances when simplifying approximations can usefully be made, whilst indicating the dangers of over-simplifying the notation. The book covers the strengths and limitations of classical thermodynamics, excluding such topics as electromagnetic, gravitational and relativistic effects (which are of little relevance to the chemist) and going beyond classical thermodynamics to discuss Boltzmann's distribution law and galvanic cells.

Symposia of the Zoological Society of London No. 44

Fish Phenology:

anabolic adaptiveness in teleosts

edited by P. M. Miller

December 1979/January 1980, xvi+450pp., £22.40/ $52.00 0.12.613344.1

The great scope of fish phenology is clearly demonstrated in the present volume. Basic topics include treatment of energy partitioning, accumulation, endocrine control, and response to environmental influences. The timing, magnitude, and fecundity of reproductive commitment is discussed for some of the many ecotypes successfuly occupied by the teleosts. A wide range of habitats from savannah pools to the deep sea are considered, as well as phenological modifications required by the migratory life-style of salmonids, seasonality in tropical habitats, and the diminutive adult size seen in many familiar aquarium fishes. Two important areas of fish phenology, not often reviewed, have also been included. These concern the nature of senescence, affecting the duration of individual anabolism, and the genetic transmission of growth and reproductive characteristics.

Na, K-ATPase: Structure and Kinetics

edited by J. C. Skou and J. G. Nørby

October 1979, xii+538pp., £22.50/ $52.00 0.12.647650.0

Since the first international conference on Na, K-ATPase was held in New York in 1973 a lot of new information has been gathered about what one contributor referred to as "this mischievous enzyme system". In order to meet and exchange views a second international conference was held in September 1978, in Denmark on the occasion of the 50th anniversary of Aarhus University. The present volume contains the papers that were presented at the meeting. In the editors' view the book gives a fair account of the present status in the field and, perhaps more importantly, it stresses the problems to be solved before it is understood how energy from the hydrolosis of ATP by this system is converted into transport work. As such it will serve as an extremely useful reference book for future work in the field, and particularly for biologists with a special interest in membranes, ion translocation and ATPase reactions.

Annual Reports on NMR Spectroscopy

Volume 9

edited by G. A. Webb

December 1979/January 1980, x+426pp., £25.00/ $57.50 0.12.505309.6

The major aim of this series is to provide timely accounts of those areas of NMR spectroscopy where significant advances are being made. In recent years the advent of FT-NMR techniques have shifted the emphasis away from ^{1}H NMR towards less receptive nuclei such as ^{13}C and ^{15}N which are now being studied routinely in many laboratories. This shift and the advances being made in the theoretical interpretation of NMR parameters are reflected in this series. In this latest volume, two aspects of the technique discussed in previous volumes are extensively reviewed and brought up-to-date, namely, the NMR of paramagnetic species and heteronuclear magnetic double resonance. Also discussed for the first time is ^{29}Si NMR and recent work on the less common quadrupolar nuclei.

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Probability and Mathematical Statistics: A Series of Monographs and Textbooks

Multivariate Analysis

K. V. Mardia and J. T. Kent

December 1979/January 1980, xvi+518pp., £26.40/ $61.00 0.12.471250.9

Broadly speaking the first half of the book contains direct extensions of univariate ideas and techniques, including exploratory data analysis, distribution theory and problems of inference. The remaining chapters concentrate on specifically multivariate problems which have no meaningful analogues in the univariate case. Topics covered include econometrics, principal component analysis, factor analysis, canonical correlation analysis, discriminant analysis, cluster analysis, multidimensional scaling and directional data. Several new methods of presentation are used. For example, the data matrix is emphasized throughout, a density-free approach is given to normal theory, tests are constructed using the likelihood ratio principle and graphical methods are used in explanation.

Proceedings of the Serono Symposia Volumes 20A and 20B

Emotion and Reproduction

edited by L. Carenza and L. Zichella

Volume 20A: November 1979, xx+684pp., £29.50/ $62.00 0.12.159401.7
Volume 20B: December 1979/January 1980, xx+734pp., £29.50/ $62.00 0.12.159402.5

The complex interactions of physiological, psychoneurological, social and cultural factors involved in human reproduction, necessitate an interdisciplinary approach. The 5th International Congress of Psychosomatic Obstetrics and Gynecology, the proceedings of which are presented in these volumes, brought together experts in all these disciplines. In addition to the more traditional topics in reproduction research, the discussions in the first volume cover fertility control, including the approaches to contraception in a number of different cultures, the psychological effects of sterilization, and the role of sexuality and certain behaviour patterns in reproduction. The second volume covers the psychological effects, both for the mother and the child, of the experience of birth; pregnancy and delivery; pain in labour; breast feeding and the bonds with the mother, and the methods of preparation and assistance at birth.

Hormones and Evolution

editied by E. J. W. Barrington

Volume 1: November 1979, xxvi+512pp., £30.00/ $69.00 0.12.079401.2
Volume 2: December 1979/January 1980, xxvi+518pp., £30.00/ $69.00 0.12.079402.0

One theme now prominent in contemporary biology is that of the remarkable adaptive diversity of organisms and the unity that underlies the evolution of this diversity. This two-volume work concentrates on research in this area, bringing together 28 internationally recognized authorities who present, in a broad evolutionary context, comprehensive and critical reviews of the information now available in many areas of comparative endocrinology. With attention concentrated on the hormones themselves, rather than on the glands that secrete them, they show where important advances are being made, and indicate the paths along which new research could profitably be directed. The wealth of authoritative and up-to-date information presented in these two volumes, and accompanied by extensive bibliographies, will make them indispensable to students, teachers and researchers in endocrinology, physiology, genetics and evolution.

Azotobacteraceae:

The Taxonomy and Ecology of the Aerobic Nitrogen-Fixing Bacteria

J. P. Thompson and V. B. D. Skerman

December 1979/January 1980, c450pp., £32.00/ $74.00 0.12.689050.1

In view of its potential economic value, research on all aspects of biological nitrogen fixation is progressing rapidly, and the publication of this thorough systematic study of the aerobic, non-symbiotic, nitrogen-fixing Azotobacteraceae, is indeed timely. The authors assembled a comprehensive collection of strains, using both local isolates and important type and reference strains from collections throughout the world, which were then examined systematically for a wide range of morphological, nutritional, physiological, biochemical and resistance attributes. The combination of the classic, systematic method with the modern, numerical analysis based on a comprehensive new set of experimental data, provides a unique treatment of this family which may serve as a model for similar treatises of other bacterial families.

Advances in Botanical Research Volume 7

edited by H. W. Woolhouse

December 1979/January 1980, xii+698pp., £35.00/ $80.50 0.12.005907.X

The aim of this series is to present authoritative statements in specialized fields of botanical enquiry which are currently in the process of rapid development. Each author places his own work against the general background of research in other laboratories and attempts to outline the possible direction of future developments, expressing his own opinions freely about controversial matters. Although the articles are primarily reviews, several in this volume contain substantial new results, for example the chapter on sodium nutrition in C_4 plants and the discussion on the rapidly-expanding subject of the physiology of orchids. These lively articles provide challenging and informative reading for all botanists whatever their speciality, at advanced undergraduate and research levels.

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The Systematics Association Special Volume No. 14

Bryophyte Systematics

edited by G. C. S. Clarke and J. G. Duckett

December 1979/January 1980, x +582pp., £40.00/ $92.00 0.12.175050.7

There is in particular a great deal of new information which has a bearing on the systematics of the bryophytes and this book, the proceedings of an international symposium, organized jointly by the Systematics Association and the British Bryological Society, examines the present state of the subject. The 22 contributions show how recent research has confirmed or changed traditional ideas about the relationships and phylogeny of the wide range of plants that compose the Bryophyta. This volume therefore presents a unique synthesis of the current state of bryophyte systematics which will not only provide a useful and much-needed reference for students, teachers and research workers, but also, because of the critical approach to this complex subject, provide a stimulus to further research.

The Genus *Penicillium* and its Teleomorphic States *Eupenicillium* and *Talaromyces*

John I. Pitt

January 1980, c500pp., £40.00/ $92.00 0.12.557750.7

This is a comprehensive and authoritative revision of the taxonomy of the fungal genus *Penicillium* — the first for 30 years. Revisions of the ascocarpic genera producing *Penicillium* imperfect states — *Eupenicillium* and *Talaromyces* — are also included. The status of all *Penicillium* species described in the literature, now numbering nearly one thousand has been assessed, including the one hundred and eighty reported since the Rapet and Thom *Manual of the Penicillia* was published in 1949. From detailed comparative studies of all known types and other authenticated cultures, together with thousands of fresh isolates, a total of one hundred and fifty *Penicillium* species are recognized together with thirty-seven in *Eupenicillium* and sixteen in *Talaromyces*. For each accepted species is provided a description taken from colonies grown under standardized conditions of medium, temperature and incubation time, in conjunction with illustrations of colony appearance and microscopic morphology. Keys to all recognized species are also provided.

Announcing a New Journal from Academic Press

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Publication: Quarterly

Subscription: Volume 1, 1980
£19.50 (UK) $57.00 (overseas)

The changing pattern of hospital acquired infection and its prevention continues to cause problems. Prevention of spread of antibiotic-resistant strains of bacteria, the increasing use of immuno-suppressive drugs, and of complex heat-labile medical equipment have all provided new problems for clinicians, nurses and infection control staff. Infection control will have to improve as legislation imposes stricter standards.

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A. Belleni-Morante

This is an introduction to the theory of linear semigroups and of linear, semilinear, and quasilinear evolution equations with particular emphasis on applications. It is intended as an introductory course for final-year undergraduate and graduate students, as well as for physicists and applied mathematicians who wish to see how an abstract theory can be used in connection with concrete problems. £9.75 *Oxford Mathematical Monographs*

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Edited by H. A . Beagley

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DIGITAL METHODS FOR SIGNAL ANALYSIS

K. G. Beauchamp and C. K. Yuen

Digital Methods for Signal Analysis is concerned solely with digital techniques in signal analysis. The book is a wholly reworked and new edition of *Signal Processing, Using Analog and Digital Techniques* (1973), which takes full account of changes since then, particularly in the areas of special-purpose data acquisition design and on-line processing systems.

The book is written primarily for people who are studying information which can be expressed as a signal, and who need to analyse the signal to produce results that are useful to their work. To this end, the authors strike a balance between theory and practice. Without requiring advanced mathematics or probability theory, they introduce the reader to the basic theoretical concepts of signal processing and demonstrate their relevance to the selection, design and analysis of computation procedures.

Dr K. G. Beauchamp is Director of Computer services at the University of Lancaster. He has been concerned with signal processing for a number of years at Cranfield Institute of Technology and the Atomic Weapons Research Establishment. Dr Y. C. Yuen is a Senior Lecturer in Information Science at the University of Tasmania.

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Observatory . . . trekking through the California high desert" and even "flying low over a Minnesota lake on the trail of timber wolves". The full weight of "the largest and most experienced staff of science reporters of any newspaper in the country" has produced journalism that is poorly researched and badly written. Surely the US national newspaper of record can produce better.

A collection by Isaac Asimov (*Life and Time;* Doubleday: New York; $9.95) is, in its own way, not much different. In place of nine reporters we have the Asimov factory — 198 books in 20 years. Asimov is a clear writer, now victimised by his own success. His *Fantasy and Science Fiction* essays are issued in book form "every 17 months", he writes for an amazing range of magazines from *TV Guide* to *Penthouse*; and he is on call to editors all over the country for articles on topics as diverse as special relativity, taxonomy, world government and space law. He is now a writer who tells his "Gentle Readers" that "my cheerful self-satisfaction is no evidence in itself that everything I write is routinely accepted". (An article on memory was rejected by *Playboy*.)

This collection, which includes cast-offs, previously unpublished works, out of print essays and duplicated material expresses little more than Asimov's "relentless determination to see that everything I have written (within reasonable limits) receives the relative permanence of book publication". Asimov has had little criticism of his work in his long career — and it shows. "I write as much as ever and the material I turn out seems to be as good as ever; not in my opinion only, but in those of my editors and readers who have no reason to lie to me".

Asimov has managed to avoid the errors of the daily journalists; he is thorough and generally accurate in reporting the facts. But his attitudes and opinions are superficial and anachronistic. There is a reflexive and unthinking defence of genetic engineering, a male chauvinist defence of the use of the word "man" in place of human, a superficial technocratic account of world politics and a nationally chauvinistic advocacy of space colonisation to be "carried through as wisely and as farsightedly as the colonisation of the American West". The Asimov of the negative solubility hoax has disappeared and he now writes as a pompous elder statesman out of touch with changing world conditions — from the job crisis in science to the emergence of revolutionary Third World political movements. His success is a success of the early 1960s and he shows little inclination to change his formula.

Joe Schwartz

Joe Schwartz is on leave from the City University of New York.

Affection for birds

BIRDS give more pleasure to humans than any other class of creature, their own kind excepted. This pleasure takes many forms and finds expression in ways which not infrequently conflict. Many who delight in birds which visit their gardens deplore those who enjoy birds caged, who in turn despise the wildfowler, who deprecates the egg-collector, who dislikes the conservationist. Thus, each forgets that he is united to the other by a bond of common interest — affection, however expressed, for birds — and that sometimes one is in another's debt.

It is an intriguing irony that the conservation of Britain's most significant contribution to the world's avifauna is entirely dependent on a section of the shooting fraternity. The red grouse — a distinctive subspecies of the willow grouse — is found only in the British Isles and would be far less numerous than it is today were it not for shooting, while the moors where it lives would be sheepwalk or sitka spruce and biologically much less interesting. In *Going to the Moors* (John Murray: London; £11). Ronald Eden traces the social history of grouse-shooting and tells something of the development of moor management and shooting techniques. In the eighteenth century, the shooting of upland game was a rugged and expensive affair — a rich man's expedition which, though the support staff might bring up supplies of cheese, sherbets and champagne, was nonetheless a serious operation. Then the romantic poets began to work their influence on good taste, which had long eschewed wild landscapes; Albert and Victoria set the seal of approval on the Highlands and suddenly the numbers of those travelling north became a multitude. In response, traders and entrepreneurs were quick to smooth the way and line their pockets. By the latter half of the nineteenth century the grouse season was part of the social calendar. Grouse management slowly became a science, designed to produce the maximum number of birds for the guns. But the grouse ceased to be a bird, a thing of beauty with a biology and behaviour of considerable intrinsic interest: for most of those involved it became a symbol and an excuse for quite unrelated pleasures: even those who actually saw and shot the birds were preoccupied with bags and performance. The book is copiously illustrated in black and white and entertainingly written to appeal to those who are or would like to be part of the grouse season society.

It is a far cry from the organised and luxurious pursuit of grouse to the unpredictable and often uncomfortable pursuit of greenshank. Desmond Nethersole-Thompson's dedicated and untiring study of this uncommon and elusive species began in 1932. With his wife, he describes in *Greenshanks* (T. & A.D. Poyser: Berkhamsted, UK; £8.80) not only their findings on the biology and ecology of the birds but also the trials and rewards that have been part of the experience. Greenshanks breed in the most lonely and desolate parts of Britain — places like the flows of Sutherland which not a few naturalists dismiss as "Mamba" — miles and miles of nothing at all. But to the Nethersole-Thompsons it is clearly a place of enduring fascination. The wildness and the wetness of this still almost immaculate wilderness are for them not the least of its charms and one must admire the cheerful acceptance of storm-bound days huddled in sleeping bags, thus saving food and fuel, until the weather improves enough to permit a return to field work. Greenshank nests are notoriously difficult to find and were once the "Cordon Bleu" of the egger: in describing the problems of nest-finding, the book conveys much of the author's own enthusiasm, and throughout there is a pleasant and rare mix of thorough scientific analysis with abiding affection for the subject. Though centred on the authors' own findings in Scotland, the book draws together other sources in Britain and Europe to form a comprehensive appraisal of current knowledge. Occasionally, the casual reader



Walking-up Grouse, by Edward Neale, 1893

Taken from *Going to the Moors*

will wish to skip minutiae but it is no disadvantage that they are included for the specialist.

Rarity and the challenge it represents appeal to many naturalists and thus they may overlook opportunities for research and enjoyment on their own doorsteps. The town pigeon lives a life of dependence on man, treating his buildings like the cliffs inhabited by its wild progenitor the rock dove and treating man himself like the cattle or goats amongst which rock doves walk unconcerned. In *The Public Life of the Street Pigeon* (Hutchinson: London; £6.95), Eric Simms takes a comprehensive look at a bird which has lived with man for well over 6,000 years. The first part of his book looks at the general biology and relationships of the order, and at the pigeon's past and present relationships with man, who pets it, eats it, races it, breeds it into bizarre forms, uses it in war and wages urban war against it, experiments with it or on it, and is photographed with it standing on his head. While grouse shooting has the social cachet and is today a not insignificant earner of foreign currency, pigeon racing outstrips it on several counts with, at Simms' estimate, several million birds kept by 100,000 pigeon fanciers and exceptional individual birds changing hands for several thousand pounds. One thing might unite these interests — a common dislike of that impartial predator the peregrine falcon. In fact, it was the pigeon racer's outcry that encouraged British conservation bodies to look at peregrine populations 20 years ago and thus discover that they were declining because of an unrecognised group of environmental pollutants, the organochlorines. The second half of the book is devoted to the street pigeon at home, its biology and behaviour, revealing how much of value and importance can be learned from the commonplace. The book contains much information, presented in succinct and entertaining style and generously illustrated in black and white.

Man's relationship with parrots is also one of some antiquity but founded on different emotions. Pigeons lack the anatomy to swagger or swear. Parrots do not provide us with a source of fresh meat in winter. The modern pigeon fancier is much preoccupied with successful breeding but the parrot keeper apparently sees his bird more as an ornament than an organism, making little attempt to provide conditions under which the birds can behave reasonably naturally, let alone breed. *Lovebirds and Related Parrots* by G.A. Smith (Paul Elek: London; £15) necessarily reflects this attitude as well as reminding us of how little is known about birds which do not occur in Europe or North America. Thus, the section on ethology occupies four pages compared with that on "simple genetics, colour mutations and colour definition" which runs to thirteen, plus an apology for compressing the subject into so small a compass. The bulk of the text summarises the pitifully little that is known of individual species in the wild. To give fair credit, the author places great emphasis on captive breeding — aviculture can no longer regard wild populations as an inexhaustible source of supply: its own depredations have had severe effects on rarer species and many others have suffered greatly from recent habitat changes. By drawing attention to problems and partial solution, the book could be a valuable contribution to the conservation of parrots.

Though scientific ornithology has only lately begun to discover much of birds' basic biology and behaviour, they were not unnoticed by previous generations, whose dialect names can provide useful insight into the earlier distribution and abundance of species. *All the Birds of the Air* by Francesca Greenoak (André Deutsch: London; £6.95) collates many, but not all, the British names. Some one suspects of being Victorian contrivances and there are some mis-attributions. The author gives a very personal view of each bird's place in lore and literature, making a pleasant if sometimes inaccurate read for the sentimental bird lover.

Christopher Perrins combines accuracy with readability in *British Tits* (Collins: London; £6.50). In the best New Naturalist tradition, the author covers the seven species of British tit, drawing together all available material from sources throughout their world range but in particular from Holland, where studies have gone on almost without a break since 1912, constituting one of the largest series existing for any bird anywhere in the world. In Britain, intensive study at a site near Oxford began in 1947 and has continued since, providing not only a direct opportunity for observation but also a comparison between the British and Continental subspecies living in subtly different woodlands. Tits are apparently a highly intelligent order, having mental and manipulative skills evolved for the survival of invertebrate eaters in a cold climate. As such, they are of particular interest in behavioural studies. The book commences with a chapter summarising the biology of each species and then goes on to examine them as a group, comparing ecological differences, food requirement, feeding habits, breeding, population dynamics and the relationship between tits, their prey and predators, respectively. The illustrations are apposite and there is an exhaustive bibliography and index. Perrins' style is eminently readable: a mass of complex information is organised with an unobtrusive precision that belies the meticulous care necessary in such work. Nor is he afraid to develop into the field of stimulating speculation an argument based on observed fact. There are still all too few such studies.

John Andrews



Coal tit (*P. ater*)

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, UK.

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Birdwatching guides

NEW bird books keep coming on the market at such a rate that it is difficult to keep up with them or to know whch ones are worth having. This review covers six smallish books which have been published in the past twelve months or so, mostly very recently. As will be seen, the books are quite disparate in their aims, so much so that comparisons between most of them are not profitable: the choice will be governed by the reader's requirements.

A Field Guide to the Seabirds of Britain and the World by G. Tuck and H. Heinzel (Collins: London; £5.25) is the most specialised of the works discussed here. This is a "standard" type of field guide, with a coloured picture, often showing more than one age group or plumage, of each species together with a descriptive text and a distribution map. The text concentrates almost entirely on identification/plumage and range. However, short sections at the beginning of each family or group amplify this with descriptions of behaviour and nesting habits. The text also contains a large number of line drawings, some of them adding additional identification features.

As already mentioned, this is a book for the *afficionado* or the globe-trotter. Although it covers almost 300 species of seabird, only a couple of dozen of them breed in the United Kingdom and several of these are rare. This edition has an extra 24-page section at the back describing British birds in greater detail with larger distribution maps for the British Isles only; apparently there will be later editions in which the resident birds of other areas of the world will be covered in greater detail.

It is a difficult task to attempt but, although it is not without its faults, it has on the whole succeeded. Both plates and text will be valuable for bird-watchers; and in ecological terms it certainly fills an empty niche. My main comment to the user would be a word of caution. The text describes differences (for example, between the shearwaters) giving the impression that the species concerned can be reliably separated in the field. Some of the characters given, such as leg colour, can be virtually impossible to see unless the bird is in the hand. In areas where numbers of closely related species occur, it takes a very experienced fieldworker to identify the birds with any degree of reliability.

Wetlands — estuaries, marshes or even reservoirs — are often rich in birds. Malcolm Ogilvie's book *The Birdwatcher's Guide to the Wetlands of Britain* (Batsford: London; £4.50) describes the main wetlands of England, Wales or Scotland (but not Northern Ireland), how to get there and what one is likely to see there. The text concentrates on the better areas in each county and on the wildfowl and waders. These two groups of birds are often present in impressive, even spectacular numbers, and in recent years, because pressures on land use have led to many 'development' plans for such 'waste' ground, ornithologists have been counting the birds on these areas. Doubtless partly because of our relatively mild winters, they have found that our wetlands harbour enormous numbers of wildfowl and waders. Birds from other areas of Europe and from the Arctic visit us in great numbers. In mid-winter British wetlands may play host to very large proportions of the populations of these birds; and therefore the conservation of our wetlands is of pressing urgency.

The book is divided by county and each major water is briefly described with information on how it might be reached. There follows a list of the most important species of wildfowl and waders together with the range of numbers which may be expected. Although more detailed figures are available to the specialist, for the casual reader these are the only figures readily available. Other species of special interest in each area are mentioned very briefly; I would have welcomed a slight expansion of this section. The book will be useful, especially to those who like to travel some distance for their birdwatching.

Peter Conder's *RSBP Guide to Birdwatching* (Hamlyn: London and New York; £2.50) is a useful little book and good value for its price. I found the title not wholly descriptive of the work. The early part is indeed a useful initiation to birdwatching, covering books, binoculars, places to go, how to keep records, and so on. However, almost two-thirds of the book comes closer, to my mind, to bird study. It covers aspects such as counting birds (usually not as easy as it may seem at first sight!), the lives of birds (flight, feathers, moult, bill, legs, and so on), migration and ringing, territory and song, nesting, distribution, habitats, ecology and food. In each of these sections the subject matter is described simply and clearly, and the ways in which the reader might make his or her own observations are suggested. There is a final section on conservation and protection, and there are useful appendices on books and other literature of ornithological societies. Useful for the beginner.

The Birdhouse Book by D. McNeil (Pacific Search Press: Seattle, Washington; paperback $8.95) contains useful sets of instructions on how to build nest-boxes and bird-feeders of a wide variety of designs. Beware, however, if you fancy the design of a nestbox, that the bird for which it is designed breeds in Britain, as the book was written for the American market. Although this is a well-illustrated book with clear construction plans, a better work for European readers is the booklet *Nestboxes* published by the British Trust for Ornithology (Tring, Herts, UK).

Discover Birds by Ian Wallace

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(Whizzard Press/André Deutsch: London; £4.95) is, as its name suggests, an introduction to bird-watching. It introduces the subject with sections on note-taking, how to identify birds, and so on. It then goes on to take the reader to seven selected bird-watching areas, such as the Isles of Scilly and the Cairngorms. For each, it describes the area fully and then the kinds of birds that one might see there. It is an attractive narrative without going into too much depth. The aim is to describe the typical birds of different areas rather than all the birds in Britain. It is lavishly illustrated by the author's pleasing line drawings and coloured sketches. In addition there are many photographs by Philip Sayer; the black-and-white ones do not always seem to have reproduced well, but the coloured photographs of the habitats are, as a group, among the best I have seen anywhere.

Alan Richards' *British Birds: A Field Guide* (David and Charles: Newton Abbot, UK; £4.95) is a departure from the standard field guide which illustrates each species with small coloured sketches designed to show the key field characters. Many current field-guides include the birds from a very large area; the British beginner must therefore sort through large numbers of species which are unlikely to occur. This book concentrates on British breeding species plus the regular winter visitors. On the whole the selection seems pretty good, though a few birds likely to be seen by the beginner get only a brief mention (for example, Bar-tailed Godwit, Great Skua, Firecrest, Rock Pipit). In this book there are two illustrations for each species, a line drawing and a coloured photograph. The text amplifies the pictures for the description and carries extra information on voice, habitat, nest and status. On the whole, this is a successful work and will be a useful addition to the beginner's library. The photographs are usually adequate for identification, but I wish fewer of them were taken at the nest, as this does not always give the right 'jizz'. For example, the waders are usually seen in other habitats where they look quite different. Also, at times, the bill shape is obscured by a beakful of insects. Apart from this, they are a pleasing collection of photographs and the general production is attractive.

C.M. Perrins

C.M. Perrins is Director of the Edward Grey Institute of Field Ornithology, University of Oxford, UK.



C.F. Tunnicliffe in his studio

Photo: Sean Hagerty

Pretty birdies

The series of books of paintings and photographs reviewed here reflects again the publishers' interest in the market provided by the ever-growing number of those attracted by birds: in the last four years over 100,000 people have joined the RSPB, bringing its total membership to over 300,000. Sadly, the contents of books published for this market are often of poor quality: either the paintings or photographs are hardly worth publishing — or buying — or else the text is ordinary, repeating *ad nauseam* the description of birds and their behaviour that can be found in the usual textbooks. Sad it is, too, that few authors whose books are reviewed here have tried to match the artist or photographer with original or more personal observations.

An example of this discrepancy is Illustrated by the *Country Life Book of Birds of Prey* (Country Life Books/Hamlyn: London; £20). Gareth Parry, a young Welsh artist still in his twenties, is a meticulous draughtsman who paints almost every barbule and barbicel. But he has not mastered the art of perspective and some birds look flat: a young kestrel really has got its underparts in a twist. Parry also falls into the habit of portraying breaks in the feather vanes, presumably to give a verisimilitude to the picture which is in fact artificial. In spite of these criticisms, however, Parry is a promising artist of the barbicel school of painting. Photographic as his pictures often are — and I am not implying that they are copied — they capture the atmosphere of the bird and its habitat; the winter paintings of the gyrfalcon on an estuary and the white-tailed sea-eagle made me shiver. The book covers all British diurnal and nocturnal birds of prey, but the vignettes by other artists seem out of place. The text by Rory Putnam is competent but it contains nothing original. The book is worth looking at but at £20 I wonder if we are not paying for the name of the publisher.

T. Lambert and A. Mitchell's *Birds of Shore and Estuary* (Collins: London; £6.95) is another coffee-table book produced for popular appeal, with just over 50 paintings of a range of sea and shore birds. Like Gareth Parry he paints in great detail but not so accurately. The colouring of the feathers, as well as the shapes of some of the birds, is so unreal as to make identification a puzzle, and it is difficult to see how some of the birds depicted flying could get their wings into the positions the artist has contrived for them. Alan Mitchell writes a short essay introducing the four groups of paintings: gulls, terns and skuas; breeding birds; ducks and geese; and finally, birds of estuaries. Each picture is accompanied by a short, popular commentary of about 300 words.

A contrast to these books is found in Réna Fennessy and Leslie Brown's *Birds of the African Waterside* (Collins: London; £12), although it is another large book of 24 paintings depicting 30 species. The paintings are warm, as befits a book on African birds. Her painting is less representational than that of Parry and Lambert and is generally very successful, although the white-starred bush robin has a badly tailored skin with wrinkles down its back — another painter's cliché which may fool the unwary. The remaining paintings are attractive studies of the waterbirds most likely to be seen on the edges of African lakes and rivers. The text is further decorated with sketches of birds and habitats. Sadly, two plates are transposed: those of the collared pratincole and the three-banded plover.

As one would expect from Leslie Brown, the text has original touches: the introduction is an account of the author's safaris up and down rivers and lakes rather than an essay on the different types of wetland, although as Leslie Brown has wandered over great sections of Africa he arrives at a good summary anyway. The text accompanying each of the plates contains more original observations and anecdotes than one usually finds in a book of this sort.

The late C.F. Tunnicliffe's *Sketchbook of Birds* (Gollancz: London; £7.95), introduced briefly by Ian Niall, is by far the most worthwhile of the books of paintings I have been reviewing. It is made up of 132 colour reproductions of pages from his early sketchbooks dating from the early 1930's to the early 1950's. The sketches are arranged in five groups: sea birds; waders; geese, ducks and swans; birds of prey; and finally miscellaneous birds. They are of value to the serious ornithologist and bird artist and will give lasting enjoyment to all who, in one way or another, are intrigued by birds. Often detailed, the feather by feather drawings, from which the later paintings were made, show changes in plumage from season to season, different feeding methods, a range of typical postures — relaxed, wing stretching and so on. Others are studies for future pictures in water colour or in crayon. On some of the plates Tunnicliffe's own descriptive notes appear. Legends give details of identification, place and date of drawing. These sketches confirm him as an outstanding artist, arguably one of the greatest bird painters of all time, ranking with or above Bewick, Audubon, Liliefors. Since the present group of sketches was taken from the earliest sketch books I would hope to see more of his later sketches. Finally, as well as being the best book in this group it is also the least expensive.

Eric Hosking's Birds (Pelham: London; £10.50; published as *A Passion for Birds* by Coward, McCann and Geoghegan: New York; $25) is an excellent title for a selection of photographs by the grand master of his profession. What Tunnicliffe was to bird painting Eric Hosking is to bird photography and, like Tunnicliffe, his photographs have stimulated the growth of interest in birds. The book consists of some 200 photographs, many of them classics, of which 80 are in full colour. It is often the reproduction of the photographs which has let this book down: some of the photographs have been cropped and enlarged to the extent that definition is poor and the rendering of the colour photographs rather pale. Eric Hosking and Kevin MacDonnel have contributed a short text in 13 chapters, a mixture of rather elementary nature notes and interesting photographic information. It is a pity that Eric Hosking did not write more about his photographic experiences. Finally, full photographic details of each photograph are given in the appendix. In spite of these criticisms it is still a very worthwhile book, with many photographs which are not only beautiful to look at but from which it is also possible to learn.

The bird photographs of Frederick Kent Truslow, a photographer well known in the US, have been treated rather differently. In *The Nesting Season* (Studio Books/ Viking: New York; $25; Elm Tree Books/ Hamish Hamilton: London; £15), the photographs are arranged in the sequence of the breeding cycle. The first half of the book, which is dominated by a popular but well written account of the nesting season by Helen D. Cruickshank, is illustrated by black and white photographs, of which few occupy more than a quarter of the page, thus giving the text more prominence than is usual in a book of this kind. The photographs lose nothing; they are all pin-sharp and seem much better printed the in *Eric Hosking's Birds.*

The second half of the 136-page book is entirely composed of colour plates, most of which are excellent but others have been over-enlarged, depicting the bird at least twice life-size, resulting a poor definition and colour reproduction. In those where the picture is given a two-page spread the middle portion of the bird is lost in the binding. Some photographs are superb, particularly the blue heron, mutual preening of moorhens unfortunately labelled "battle for dominance", and the American national bird — the bald eagle — has a striking portrait. Unique are three photographs showing a pileated woodpecker carrying its three eggs to a new site after the top of the nest tree had broken off, exposing the nest. An appendix gives some of the photographic data but lacks details of film stock and camera.

The five volumes published by Orbis entitled *Birds of Ocean and Estuary, Birds of Mountain and Moorland, Birds of Marsh and Shore, Birds of Heath and Woodland* and *Birds of Hedgerow and Garden* (Orbis: London; £7.50 each) have made two previous appearances in Europe as a part-work, first in Italy and then in Britain under the title *The Orbis Encyclopaedia of Birds of Britain and Europe.* In Italy it was nothing more than an encyclopaedia of European birds, describing 642 species in systematic order with poor paintings of birds — nearly all the beaks, for instance, were too small, the colours were not always representative and often the shapes are inaccurate. For the English part-work John Gooders made the best of a bad job and wrote a number of popular essays on various aspects of a bird's life, added many excellent photographs and, particularly useful, colour plates of the wing patterns of nearly every group of birds. This third version is the same English part-work now in five hard-backed volumes with the misleading titles given above. The titles are misleading because as the cover admits in small print, the work lists the birds in systematic order. Consequently, the first volume, entitled *Birds of Ocean and Shore,* includes most ocean or shore birds but omits waders, passerines and so on which regularly visit the shore. The same criticism can be made of the other volumes. *Caveat emptor* applies and the £37.50 for the five volumes might be better spent elsewhere.

Peter Conder

Peter Conder is a writer and a wildlife management consultant who has recently been working for IUCN/WWF in Pakistan and Jordan.

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Invertebrate Neuropharmacology

Lucy D Leake
Robert J Walker

The invertebrates, with their less complicated nervous systems, are being increasingly used to model the neuronal activity of vertebrates. In reviewing progress in the field to date, the authors of this book highlight the benefits and shortcomings of the applications of invertebrate studies to the vertebrates, and indicate possible future developments. A comprehensive, current bibliography is an important feature of the book.

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Wildlife in danger

THE conservation band-wagon trundles on, dropping a seemingly endless shower of new books about endangered species and their approaching extinction. One may wonder how many hundred square miles of virgin forest are clear-felled every year to make pulp for the paper on which they are printed, and what happens to the inhabitants, plant and animal, of the biome destroyed. Some of the books are worth the sacrifice, but as for others — less talk might result in more conservation though fewer royalties for the riders on the wagon.

Australian Endangered Species (Cassell: London; £15) by Professor Derrick Ovington, formerly Professor of Forestry at Canberra and now Director of the Australian National Parks and Wildlife Service, is one of the best books on the problems of the conservation of nature to have appeared for a long time. Although it deals specifically with the fauna of Australia, much of its information is applicable to any part of the world. The author writes as a scientist and deals with his subject objectively without sensationalism, and the resulting clear and sympathetic account of the history, present state and future prospects of the Continent's fauna and flora makes fascinating reading. The early white pioneers, isolated in an alien world and facing incredible hardships, understandably felt an overwhelming urge to subdue and transform the wilderness in order to survive. Many species of the fauna were abundant and were killed for food or recreation, or, when farming was established, as vermin. The early settlers did not understand that breaking-in the wilderness for agriculture or stock-raising so altered the environment that some native species could not survive. Furthermore they could not foresee the dire results of the well-meant introduction of alien species for practical or sentimental reasons. The extinction or extreme rarity of many species is attributed in frequent instances to habitat alteration by stock or rabbits, and to introduced predators such as the cat and the fox, so that species once widespread and abundant have suddenly become scarce or disappeared. "Like fresh water or clean air, wildlife is liable to be taken for granted and its value underestimated until supplies run short".

The heart of the book deals with the 23 Australian mammal, 18 bird, and 2 reptile species that are regarded as endangered. Each species is depicted in a full-page coloured illustration facing a page with its description and biological details, and a map of former and present distribution; the pictures are beautifully done and well printed. The book concludes with an account of what has been done, and what remains to be done, in conserving Australian wildlife, emphasising the need for massive further research and for education: "the dissemination of information about wildlife to many people and agencies is essential if sympathy with wildlife protection measures is to be translated into active support and adequate funds are to be made available." This is a splendid book that will be valued by specialist and layman alike.

The Living Swamp (Orbis: London; £5.95), by A. Borgioli and G. Cappelli, describes the different kinds of marshes, swamps, fens and bogs that occur throughout the world, and illustrates their flora and fauna with many excellent colour photographs, all of them beautiful and many dramatic. The first of the four chapters deals with the aquatic environment and explains the origin and evolution of marshland, pointing out its inherent instability under the influence of natural forces as well as under the activities of man. Many are threatened with destruction through efforts to reclaim wastelands for agriculture or other purposes, such as the construction of airports for commerce or marinas for amusement. The second chapter displays the "marsh microcosm" and the food chain, ranging upwards from unicellular algae to vertebrate animals, based on the rich primary production of organic material synthesised by plants. The illustrations of this chapter include many unusual photographs of invertebrates, mainly insects, and excellent pictures of amphibians and reptiles. A well illustrated chapter on birds, "the lords of the marsh", then follows, pointing out how few mammal species compared with the host of birds are able to take advantage of this environment. The final chapter describes the characteristics of the major marshlands of the world and lists them by countries, noting the national parks and reserves where conservation of this specialised habitat is practised. The text well matches the illustrations to make the volume more than merely a coffee-table book; it has, however, suffered here and there in translation from the Italian, particularly with names of species, as where it mentions "greylag" geese in Argentina.



White-fronted lemur, San Diego Zoo

Taken from *Lifeboats to Ararat*

In 1903 certain sportsmen, fearing that the stocks of big game that they hunted were diminishing, founded a Society for the Preservation of the Wild Fauna of the Empire, with the object of establishing game reserves and preventing the natives poaching the animals the alien whites wished to kill; no wonder the popular press lampooned them as "penitent butchers". Mr Richard Fitter has adopted the nickname as the title for his short history of the society (*The Penitent Butchers: 75 Years of Wildlife Conservation*; Collins/Fauna Preservation Society); Sir Peter Scott has done the drawings. Wildlife protection has always begun as an attempt to preserve game animals from overshooting or overhunting: "one of the few amenities that could be offered to soldiers (officers only, of course) and administrators marooned in darkest Africa . . . was shooting big game." The move from the protection of game to the protection of all wildlife came slowly, and it was not until well after 1945 that the conservation of whole ecosystems gained acceptance. The author devotes a chapter to breeding rare animals in captivity in order to return stocks to the wild, a measure that has had several successes, but is a last desperate remedy that for many reasons cannot hope to be repeated often.

The difficulties of breeding rare animals in captivity to save them from extinction are discussed in some detail by Sheldon Campbell in *Lifeboats to Ararat* (Weidenfeld and Nicolson: London; £6.50). This book, by a former zoo-keeper, is a chatty anecdotal account of life "behind the scenes" in zoos round the world and particularly in the San Diego zoo of California. The author says, "no activity by zoos has received more lip service and been less seriously undertaken than captive breeding. All zoos subscribe to the idea, but few are really doing much about it." A major obstacle to success in returning captive-bred animals to the wild is the difficulty of gettting them to earn their living when they have known only the soft life of a zoo "welfare state". The difficulties are such that the author concludes: "the probability is that zoos will hold many captive-bred species far into the foreseeable future, and, possibly, some for ever."

The truth of this statement is well illustrated by Peter H. Beard in *The End of the Game: The Last Word from Paradise* (Collins: London; £10), an unusual but interesting and instructive book. It is the story of the elephants of the Tsavo region of Kenya, teeming with wild animals at the beginning of the century, but which, after being made a National Park, degenerated into an empty desert strewn with dead trees and the skeletons of the elephants it was intended to preserve. When the wardens and rangers of Voi Force finally stamped out poaching in the Tsavo Park in the 1950s they unwittingly sealed the fate of Tsavo,

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Karen Blixen and Ingrid Lindstrom, Ngong, 1921

Taken from *The End of the Game*

for the elephants multiplied, and when the great drought came they destroyed their habitat and perished in the wilderness they had made. The book is lavishly illustrated with photographs of animals and men from the early days of colonial settlement until the present, a fascinating collection. The coming of the white man who built a railway and imposed his will on the land and its inhabitants was "attended by glory and courage, ennobled by sacrifice, enriched by science, medicine and law. But it marked the beginning of the end in a land where nature herself had always been sovereign . . . It is too late to undo what has been done . . . we have conquered nothing at all."

L. Harrison Matthews

L. Harrision Matthews was formerly Scientific Director of the Zoological Society of London, and a member of the scientific staff of the Discovery Expedition, 1924-28. He has also done zoological research in the Arctic, South America, and Africa.

Making a living

THE word 'predator' immediately conjures up visions of a lion tearing at a carcass or of a peregrine falcon swooping on to its prey, but smaller and more numerous predators are at work everywhere if only we look for them. There is all the drama of the predator and its prey when a wasp butchers a butterfly or when a dragonfly pursues its insect victim over a garden pond. *The Hunters* (Hamlyn: London and New York; £5.95) by Philip Whitfield takes an imaginative and exciting new look at the world of predators and will inspire student and layman alike. It covers the whole range of hunters from tiny single-celled protozoa to large mammalian species. There is an introduction on population numbers and food chains and then a series of chapters that group predators together under the methods they use for hunting.

Tigers, praying mantis and chameleons all hunt by stealth, relying on concealment to enable them to get close to their quarry. Some animals hunt in groups like soldier ants, pelicans and hunting dogs. Others, such as burying beetles and vultures, scavenge for dead animals. Ciliate protozoa, flamingos and baleen whales all use filtering mechanisms to catch their aquatic prey. There are also chapters on predators that use tools or traps and those that are specialised in the food they eat or in their methods of detecting prey. Every predator is given a double-page spread of text and diagrams which explain its hunting method; and the book is illustrated with some superb drawings by Richard Orr and Michael Woods. These include a walrus disembowelling a narwhal, an anemone engulfing a fish, a boa constrictor crushing an ocelot and a brown bear sweeping a salmon out of a torrent.

These two artists have produced some more beautiful and informative diagrams and drawings for another book in the same series, *The Animal Family* (Hamlyn: London and New York; £5.95) edited by Philip Whitfield. This looks at the diverse ways in which animals find mates and rear their young. Like the previous book, it illustrates various topics with reference to examples chosen from the whole Animal Kingdom. The mechanics of sexual reproduction are explained, including the structure and function of the reproductive organs, hormones, fertilisation, and the strange animals that change sex. There are clear diagrams illustrating the variety of visual and vocal courtship displays, territorial behaviour and nest sites. Some examples of family life are considered in detail, such as the caste system of social insects, the complex social relationships in monkeys and apes, and parental care in crocodiles.

It is a pity that the text suffers so badly from the fact that several authors have contributed to the book. In one chapter the selfishness of courtship is rightly emphasised together with the conflict of interests between male and females and the diverse ways in which males compete with each other for the opportunity to mate. In the next chapter, however, we read all about harmonious pair bonds and are told that sneaky male mating strategies are not favoured because this would be harmful to the population. We even go right back to the old, and surely wrong, view that territorial behaviour exists to ensure that the population never grows too large and that fighting is ritualised for the good of the species.

The migration of animals has astonished man since the earliest times. The first explanation of the seasonal emigration of some species and the simultaneous appearance of others was the transmutation hypothesis. In summer, robins were supposed to change into restarts and hawks into cuckoos. Swallows were imagined to sleep all winter in the mud at the bottom of ponds while geese were thought to grow from barnacles. We now laugh at these old ideas but in *Animal Migration* (Orbis: London; £5.95) John Cloudsley-Thompson shows that the true explanations can be just as strange.

Tiny warblers flicker by night across thousands of miles of desert and ocean; spiders balloon about the skies on threads of silk; and salmon return from the sea to the exact streams in which they were born. There is a chapter on how the Sun, the stars and the Earth's magnetic field are used for navigation; and then the bulk of the book is divided into three sections: migration by air, land and water. Examples from all over the world are described with a clear and lively text, illustrated by abundant drawings, maps and colour photographs. All extremes are considered from the few centimetres that limpets wander round their



L. Harrison Matthews and Desmond Morris at London Zoo

Taken from *Animal Days*

home base on a rock to the vast wanderings of the shearwater over the polar seas.

There are good accounts of the migration of large mammals, the movements of plankton, fish, turtles and whales in the sea, and of bird, bat and butterfly migration in the air. It is emphasised that animal movements on such a vast scale, without respect for international frontiers, present serious problems for conservation. What is the use of restricting whale kills in one part of their range if the restrictions are violated when they migrate elsewhere? The book is an excellent portrayal of the wonders of migration but has little to say on its evolution or adaptive significance which is dismissed as "one of science's many unsolved mysteries". Migration is said to be a widespread "instinct" of advantage to the species, which obscures the important point that different individuals within a species may migrate in different ways. Readers are referred to Robin Baker's recent book, *The Evolutionary Ecology of Animal Migration* (Hodder and Stoughton: London, 1979) for an up to date account of this problem.

In *Colour for Survival* (Orbis: London; £6.95) Peter Ward looks at the ways in which colour influences animal behaviour and ecology. The book is illustrated with spectacular colour photographs most of which are by the author himself, who has travelled widely in Africa, Asia and Central America and has first-hand knowledge of the animals he describes. It gives us an exciting insight into the use of colour in animals for camouflage, mimicry, warning and display. There are some marvellous examples, such as the caterpillar that looks like the seed-packed excrement of a frugivorous bird and another that resembles a feather stuck to a wet leaf. The diverse uses of colour are described in insects, lizards, frogs, birds and large mammals. My favourite is the flower mantis from Borneo which has bright white petal-like extension of the body that lure flower visiting insects to their unsuspecting death.

Animals and their World (Blandford: Poole, UK; £8.95) by Mary Parker Buckles, which is subtitled "The astonishing story of how mammals live in their own ecological zones", left me mystified rather than astonished. The book is divided into chapters which deal with the major habitat types such as tropical and temperate forests, grasslands, tundra, desert, and the oceans. Every chapter begins with a brief ecological description and there then follows an idiosyncratic list of some of the mammals of the habitat with photographs and brief notes on each species. There is little relationship between the examples given and the ecological comments at the start of the chapter, and no attempt has been made to show how the different species are adapted to their various habitats. Because the book restricts itself entirely to mammals, there is no detailed account of how plants and other animal types influence mammalian life-styles and the reader is left in a sea of examples wondering what to conclude.

A second book which uses the same plan, but with great success, is *The Living World of Animals,* another outstanding publication by the Readers Digest Association (London; £9.95) in association with the World Wildlife Fund. The main part of the book is devoted to sections on the different ecological zones of the World and shows, with an authoritative text and an abundance of excellent colour photographs and diagrams, how animals make a living in their various environments. There are also chapters on animal distribution, evolution and behaviour, and relationships with man. The book manages to convey the beauty and magic of the natural world, from the cold white silence of the polar regions to the richness of the tropical forests, and at the same time gives an up-to-date account of our knowledge on animal adaptations. As such it must be one of the best natural history bargains on the market.

Desmond Morris has spent most of his life surrounded by animals, from student days with a laboratory that was filled with dozens of fish and birds to the years as curator of mammals at London Zoo where his office was a cage with a desk inside! In *Animal Days* (Jonathan Cape: London; £5.95) he recalls the people and events that have influenced his life. The result is a book that is great fun to read, a mixture of rich observations on animals and stories that are always amusing and sometimes hilarious. With varied interests that included film-making and paintings as well as Natural History, it was lectures by Niko Tinbergen and Konrad Lorenz that finally inspired Morris to take up zoological research. Baptised and then confirmed by these two great fathers of ethology, he moved to Oxford to study for a doctorate under Tinbergen. Here he made some fascinating observations on homosexual behaviour in sticklebacks and divorce in finches. He reared a pet crow that invaded nearby gardens and learned to squawk "Go away" in the cultured accent of an Oxford don. There were amusing meetings with J.B.S. Haldane and Julian Huxley and a wonderful story about Lorenz and a tame raven which I will not spoil for the reader.

Later on, in London, the author started the successful weekly television programme 'Zootime'. In front of the cameras the animals did not always behave as expected, like the time that the giant tortoise, roused to great activity by the warmth of the television lights, charged around knocking over the cameras and crew. The days at the Zoo also showed how sometimes hand-reared, captive animals could be reduced to hopeless mental hybrids, like the panda Chi-chi who preferred humans to her Russian spouse An-an. The famous artistic chimpanzee Congo and Guy the gorilla also rejected their own species as mates. Ethological research has helped us to understand the social and environmental needs of different species and Morris emphasises that modern zoos must provide these so that we can see the animals behave in their normal way.

There is, however, no substitute for observations in the field, although this can often be a time-consuming and frustrating occupation, as Alan Goodall shows in *The Wandering Gorillas* (Collins: London; £6.50). The book is mainly about the wanderings of the author in central Africa and the difficulties of life in the forest although we do get some occasional glimpses of the elusive gorillas themselves. By behaving like a gorilla, chewing leaves and beating his chest, Goodall was sometimes able to observe them at close range. On other occasions he experienced the terrifying bluff charges where a male would stop his advances only a couple of metres away! The gorillas are threatened by extinction, partly from local superstition (one group of six was stoned to death) but mainly by destruction of their natural habitat. Alan Goodall concludes with a plea for international aid to enable the central African governments to offset short-term economic losses and leave the forests intact so that these gentle giants are able to continue their wanderings in peace.

Nicholas Davies

Nicholas Davies is a Demonstrator in Zoology and Research Fellow of Pembroke College at the University of Cambridge, UK.

Intricacies of Nature

THERE seems to be no end to the flow of new books by experienced naturalists whose purpose is partly to educate the public in the intricacies of nature and partly to provide a forum for their authors' reminiscences. *A Natural History of Britain and Ireland* by Eric Simms (Dent: London; £6.95) has a flavour of the nineteenth century about it — accentuated by Robert Gillmor's exquisitely drawn vignettes at the head of each chapter. The author describes the very varied countryside of Britain in terms of its history and of its geographical differences. The book is divided into 15 chapters each of which deals with a different region that typifies a distinctive type of habitat. The book is also largely biographical, recalling the author's many experiences of wildlife in different parts of Britain. It does not make very easy reading — many of the accounts of specific localities, such as the Cheddar Gorge, consist largely of lists of species that the author has seen there. So much information is presented that the underlying theme of the book, an analysis of the factors that have produced the natural history that we see today, is frequently submerged.

Also by Eric Simms, *Wildlife Sounds and their Recording* (Elek: London; £5.95) goes in great depth into one aspect of natural history study, the recording of animal sounds. The book deals with detailed techniques and descriptions of recording equipment, the history of wildlife sound recording and something of the biological significance of auditory communication in animals. There is much interesting information about techniques and about the kinds of sounds that animals make. Unfortunately, the chapter on biological aspects of animal sounds is rather dated. In particular there is little mention of the highly fruitful use of playback experiments in which recorded sounds are played to animals in the field. This has been notably successful in increasing our understanding of the nature of territoriality in birds, competition between red deer stags, and mating strategies among frogs and toads, where synthetic sounds have proved very useful. The book contains a number of interesting photographs, particularly those of early recording equipment.

Encounters with Nature by Leslie Brown (Oxford University Press: Oxford; £6.50) is more revealing of the author than informative about wildlife. Consisting of a series of essays about encounters with animals that the author calls "golden moments" in his life, there are chapters on the aardvark, badgers, flamingoes and pelicans among others, seen mostly in East Africa and Britain. Some of the observations of animals in their natural state are evocative, exciting and informative but too much of the book is devoted to the author's opinions about the state of the world as he has found it during his life. The book is introduced by a series of contemptuous remarks about "scientists" and is punctuated throughout by comments that reveal that Leslie Brown yearns for the good old days of the British Empire when natural history was a hobby for a few privileged amateurs. While reading this book one inevitably finds that its rather sour, misanthropic tone contrasts with Gerald Durrell's much more amusing and sympathetic treatment of humans as well as animals. The book includes a series of attractive drawings by Doris Tischler.

In marked contrast to Brown's book, with its emphasis on more exotic and spectacular forms of wildlife, Ken Hoy's *On Nature's Trail* (Mitchell Beazley: London; £6.95; ALW: New York; $14.95) brings us back to the minutiae of wildlife in our immediate surroundings. This excellent and attractive book is a practical guide, telling the reader what to look for, where to find it and how to interpret what we find. It tells us what we can learn from animal droppings and tracks, what we can do to help injured or orphaned animals, how we can appreciate the complexity of life within a single tree, and much more besides. The book is generously illustrated with excellent photographs. As well as making a very good companion for any young enthusiast, this book should prove useful to teachers in search of ideas for nature trails and school field projects.

Another immensely useful book is *A Day in the Country* by John Gooders (André Deutsch: London; £5.95). This provides a pocket guide to naturalists and more casual observers of wildlife, telling them what they might see in any part of Britain. The book is divided into two parts. Part 1 lists all the National Parks, Forestry Commission areas, RSPB reserves, and many other official localities. Clear maps and lists of addresses make the planning of visits to such places a simple matter. Part 2, which takes up the greater part of the book, deals separately with eleven areas that together cover the whole of Britain. For each area there is information about footpaths, areas of outstanding natural beauty, wildlife parks and zoos, stately homes and many other places of interest to the naturalist. For each locality there is a list of the more interesting and unusual animals and plants that the visitor might expect to see. Illustrated by a number of very attractive sketches by David Thelwell, this book makes a truly valuable contribution to the enjoyment and appreciation of Britain's wildlife.

Tim Halliday

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Wildlife of Europe

THE quartet of books reviewed here ranges from the broad canvas of the classification of the Animal Kingdom to narrower studies on groups and single species of carnivores. Naturalists will find the first useful, the rest diverting as well as informative.

Mammals: Their Latin Names Explained by A.F. Gotch (Blandford: Poole, UK; £5.95) clothes the dry bones of taxonomy for the benefit of those without a classical education. The scientific names of animals are, on the whole, indicative of their appearance, provenance or discoverers, and much is hidden from those who cannot derive the roots from which the names are composed. Thus the New World cottontail is known to zoologists as *Sylvilagus floridanus* which tells us that it is a 'woodland hare from Florida'. English names vary locally and lack the diagnostic precision of the scientific name.

The author of this book deals first with the phyla and subphyla of the whole Animal Kingdom from *Amoeba* to man, then with the vertebrates, down to orders in the case of mammals, and finally — the bulk of the book — with mammals down to families and selected species. The principles of nomenclature are explained, although it is surprising that the importance and adequacy of the first published description of a species are not more emphasised. At every step the Latin or Greek derivation of names is carefully expounded and — particularly valuable — a biographical note is given about explorers and scientists, after whom many animals are named. There are a few misprints and the odd contentious derivation; for example, I do not believe that the name *Didelphis* for the opossum refers to the pouch as a "secondary womb" (page 37): surely it indicates the double nature of the female reproductive tract in marsupials. In any case *Didelphis* does not have a pouch.

This book is a mine of information and illumination to naturalists who have used these names for years without understanding their import.

Carnivores of Europe by Robert Burton (Batsford: London; £8.50) is a timely evaluation at second hand of the status and prospects of the terrestrial, carnivorous mammals of Europe. These most interesting animals have been subjected to intense persecution by man, both as competitors for his domestic stock and as providers of warm and elegant clothing. Now that predatory animals are acknowledged to play a salutary rôle in the economy of nature, Mr Burton's book, giving up-to-date results of recent researches, helps us to assess probable future trends in numbers, both down-



Eris Simms with reflector microphone

Taken from *Wildlife Sounds and their Recording*

wards, due more now to fragmentation of habitats than to direct killing, and, surprisingly, upwards because of ill-judged introductions — for example, American mink and raccoon dogs. In addition, we have to face the possibility that extremely adaptable species, for example, the fox, are now flourishing in urban and suburban environments and could become a menacing carrier of rabies, which would be far more serious than the presumed agency of the badger in transmitting bovine tuberculosis.

The predator which seems to have the least hope of avoiding ultimate extermination, is the wolf. B.H. Lopez in his book *Of Wolves and Men* (Dent: London, £7.95) has produced an impressive and extensively documented survey not only of the natural history of this species but also of the significance of the wolf in hunting societies and of the bitter enmity that exists to this day between it and pastoralists. Most of the book is the outcome of tireless research among the literature but the author has been at pains to spend long periods in the field with Esquimaux in Alaska and to come to an understanding of how wolves are an intimate part of their world. If the wolf has to go, then this book will compose a worthy obituary.

Lastly, we have *Lutra, the Story of an Otter* by Philip Wayre (Collins: London; £3.95). This is the latest in the series *Collins Animal Lives*, each written by a specialist but with a presentation and an outlook which make easy reading for the layman and children. Because the otter is a declining species in England now, much sound and fury has been raised by protectionists to abolish otter hunting and to declare it a protected animal. The results of recent fact-finding surveys suggest that powerful causes of the otter's decline are pollution of our waterways and the increasing pressure of man's activities on them; in which case the establishment of the Otter Trust in Norfolk, of which Mr Wayre is Honorary Director, and the creation of quiet areas as refuges hold out the best hope of rehabilitating this very attractive animal. Mr Wayre's little book, which describes a year and a bit in the life of a bitch otter in East Anglia and is enlivened by the delightful sketches of John Edwards, will make many of his readers wish to do their bit in conserving the species.



Common hamster

Taken from *Animals and their World*

H. N. Southern

H. N. Southern was until his recent retirement Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford, UK.

Seal saga

DURING the past century the grey seal has increased in numbers in British waters mainly because of the decrease in seal hunting by man. In the same period the status of most stocks of commercially valuable fish in the seas off the Scottish coasts, where the grey seal is most numerous, have declined due to overfishing by man. Seals and man both eat fish, but the quantity and quality of the seal's diet is, for a variety of technical reasons, inaccurately known. Assuming a grey seal eats 7 kg fish daily there is a postulated loss of some 56,000 tonnes per annum to the British in-shore fishery, estimated at £15 million. Unfortunately, the science of both the fishery and seal conservation aspects of the issue are riddled with assumptions and untested hypotheses.

These are the ingredients of a roaring conflict which has arisen and has already been staged between fishery and nature conservation interests in Orkney and North Rona during the breeding season of grey seals in 1978. A thoroughly objective review of the whole affair is difficult to obtain, firstly because of the formidable dossier of evidence and often conflicting expert comment and secondly, because of the emotive aspects of killing animals of such immense human appeal as seals. Nonetheless, in *Seal Cull: The Grey Seal Controversy* (Penguin: Harmondsworth, UK; 95 pence), John Lister-Kaye has come close to providing an account which is fair to both sides.

Lister-Kaye has provided a first class documentary of the grey seal-fishery issue in which he has systematically presented the information in a form which is useful to statesman, politician, scientist, fisherman, conservationist and interested layman alike. He writes not as a completely detached observer and is not afraid to state his own opinion on main issues and attribute blame or praise where he thinks fit. He also gives thought to the primaeval roots of the problem in "man and dog" relationships and very sensibly relates the conflict to the international scene and to harp seal harvest in Labrador from which the grey seal issue in Britain derives impetus. A set of personal interviews with prominent personalities shows them to be to a great extent in ignorance and disarray on major aspects of the controversy.



Seal pup

Taken from *Seal Song*

In conclusion there is a personal view in which the author, though hard hitting in some respects, does not damage his balanced treatment. The book does nothing but good in suggesting guidelines by which a compromise in grey seal conservation can be achieved.

In 1978 Orkney was a focus of national public interest because of the cull of grey seals there and at North Rona. In the arena Greenpeace and a local action group called 'Selkie' (Orcadian for 'seal') were pitted against Norwegian seal-hunters who were the contractors brought in by the then Secretary of State for Scotland, Bruce Millan. For every contestant in the field, there was at least one media man. The prospect of shooting, blood and confrontation brought the reporters and photographers in a swarm to Kirkwall.

Sue Flint, a seasoned campaigner for good causes in Orkney, was a moving spirit in the local Selkie group and painstakingly

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DNA TUMOR VIRUSES
Part 2 of Molecular Biology of Tumor Viruses

Monograph 10B

Edited by **John Tooze,** *European Molecular Biology Organization*
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February 1980 LC 79-19882 CIP

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Contributors include: N.H. Acheson, S.J. Flint, B.E. Griffin, T. Grodzicker, B. Padgett, B. Roizman, P.G. Spear, P. Tegtmeyer, H. zur Hausen

Appendixes containing the following DNA sequences: SV40, Polyoma, Human Papovavirus BKV, and Adenoviruses 5, 7, and 12 were contributed by A.R. Buchman et al., B.E. Griffin et al., I. Seif et al., J. Maat et al., R. Dijkema et al., and H. Sugisaki et al.

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Part 3 of Molecular Biology of Tumor Viruses

Monograph 10C

Edited by **Robin A. Weiss** and **Natalie M. Teich,** *Imperial Cancer Research Fund Laboratories*
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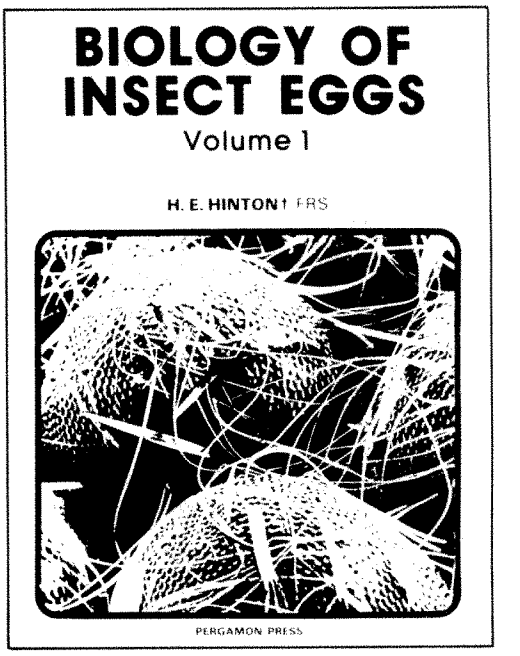
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wrote a diary of events which appears in *Let the Seals Live!* (Thule Press: Sandwich, Shetland; £5.50). This is the story from the Selkie side of the action which, understandably, portrays their action as verging upon the heroic. It is primarily about people, secondarily about seals. If Mrs Flint did not write with such a light touch and with such humour and feminine charm, the book could be a tedium of trivia enlarged to assume an importance far beyond reality by the ambient tension which gripped Orkney at the sight of the Norwegian sealer, *Kvitungen,* closely shadowed by Greenpeace's vessel, *Rainbow Warrior.*

However, much more can be read from this book than a faithful account of a local and national campaign to save the Orkney and North Rona seals from slaughter. It shows clearly the delicate balance which is struck between the rational behaviour of ordinary people and that of the same moving on a sense of bitter outrage. It also shows that the same local people can expect strong national and international support, not just in words but in action on the spot, in very difficult conditions. It also shows how extended, exhausted and near to collapse was the local expeditionary force. The book has many mistakes and shows all the signs of having been rushed. It is, however, something of a latter-day Orkney saga — both informative and entertaining.

Seal Song (Allen Lane/Penguin: London and Harmondsworth, UK; hardback £4.95, paperback £2.50) is a sensitive portrait of the harp seal created in words by Brian Davies with photographs by Eliot Porter. Davies is the executive director of the International Fund for Animal Welfare which has been, through his personal energies, responsible for the campaign against the annual hunt mainly of harp seals off Newfoundland and Labrador.

The book, produced in 'coffee-table' style, described the author's commitment to the battle against the cruelty inflicted by mankind upon wildlife for economic gain. He finds in the harp seals the quintessence of natural beauty, evolutionary adaptation and a subtle psychological, perhaps mystical relationship between man and beast. His reactions are instinctive of a person who is repelled by any sense of pain or bloodshed and there could not be a more vivid subject for such reactions than the slaughter of the fluffy, white seal pups and their sleek, silken dams in the crazy, white world of the sea-ice.

Inevitably, therefore, this is an account which is heavily charged with emotion; while providing some salient scientific information and assessment of the social and political issues, the author makes a direct case for the cessation of the seal hunt on grounds which are mainly humanitarian. His fears for decimation and possible extinction of the harp seal are, however, very real when seen against the potential for exploitation embodied in a sealing vessel such as that shown in this book.

There are compelling descriptions coupled with excellent full-page colour photographs of the harp seals both on the ice floes and in the strange phantom light in the sea under the ice. Porter's photographs provide superb contrast in texture; the soft, downy warmth, black muzzle and watery, wide-awake eyes of the pups seen against the hard angular shapes of the sea-ice.

The book concludes with a personal account of efforts made by Davies and others against great odds to fly the world's press to the scene of the hunt, in which he risked his life. An outstanding piece of publicity against the seal hunt, it is short, easily read, lavishly illustrated and has instant impact.

A seal emerging from the sea is like a stranger from another world; yet for a few crucial days each year the grey seal must come ashore above high water mark to give birth and mate. They are then so vulnerable to hunters and available for study by scientists. In the 1930s the late Sir Frank Fraser Darling started the scientific work in the Hebrides and this was continued after the war by Dr Leo Harrison Matthews and his friends Professors E.C. Amoroso, Richard Harrison and the late Humphrey Hewer. They became fascinated by the behaviour, reproductive anatomy and physiology of both the grey and common seal and spent many enjoyable days in the field and laboratory piecing together their biology, particularly the evidence of delayed implantation of the blastocyst, possibly related to the spring moult in the grey seal.

The story is told in the style of a biographical novel by Harrison Matthews in *The Seals and the Scientists* (Peter Owen: London; £7.50). A series of conversation pieces with his zoologist companions in strange places are interspersed with contrasting descriptive sketches of the Welsh Islands and the Wash. There are some exciting moments in coming face to face with the heroine — a grey seal cow called Mrs Mopp!

The discussions of the academics are full of accurate scientific information presented systematically in informal guise. However, the book deals only with the early years of a period of great expansion in seal biology in Britain and the relationship of seals to sea fisheries. That a book of this title and character should have been published in 1979 without greater mention of the seals and fishery issue and some of the main personalities (because it is about people as well as seals) is a great pity. Though sprinkled with many technical and scientific terms, the book will be enjoyed by both naturalist and layman.

J. Morton Boyd

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Living with cetaceans

SCIENTISTS have seldom been able to study living cetaceans directly, but have usually had to work like detectives building a story either from clues washed up on the beach or from inspecting victims of the whaling industry in order to infer something of their lives from corpses. Unfortunately, the popular tradition of celebrating the careful deductive approach of the detective hero has not become established among popular books on cetaceans. Instead, the cetacean literature has suffered from authors who have taken advantage of the lack of knowledge about these mammals to speculate freely and to present these speculations as the truth.

Whales, Dolphins and Porpoises by Ronald Lockley (W.W. Norton: New York; David and Charles: Newton Abbot, UK; $14.95, £6.95) is a case in point of this unfortunate tendency. The book is written in a careless, chatty style and its organisation is confused. Many sections seem to have little to do with their headings. For example, the section entitled "Anatomy and physiology" concentrates on cetacean intelligence, bouyancy, diurnal rhythms and sleep. The rough draft quality of this book is by no means as harmful as the fact that many statements are either confused or false; this problem is aggravated by the fact that not all references quoted in the text are listed in the bibliography.

Lockley's evaluation of the cetacean literature is typified when he maintains that dolphins can probably perceive our thoughts by telepathy and that baleen whales are known to echolocate; or when he states: "They [cetaceans] display almost every human emotion you can name — in short, cetaceans are almost human.". Lockley commits enough serious errors of fact that I cannot recommend this book to anyone who wants to be able to distinguish what is known about cetaceans from what might be true or is simply false.

This is a shame, for the book contains many beautiful and interesting photographs as well as a useful set of descriptions and illustrations of most species of cetaceans. Lockley's account of interactions between humans and cetaceans, both cooperative and predatory, is detailed and is much more sound than his attempt to describe cetacean biology.

Wake of the Whale (Hutchinson: London; Friends of the Earth: New York; £15.95) is not a book about whales, but rather describes the work of one of the finest photographers of whales, Bill Curtsinger. The book is organised around a collection of superb photographs taken

by Curtsinger of marine mammals around the world. The large format of the book enhances the fine reproduction of these photographs, which comprise half of the book. Wild cetaceans are among the most difficult of photographic subjects, and this difficulty tells, for the pictures of cetaceans in this book cannot compare with those of seals — either in composition or clarity. In part because of these difficulties, this collection of photographs is eloquent evidence of the persistent and devoted skill of Curtsinger as a photographer.

The text accompanying these photos exhibits a curious ambiguity of purpose. Not only does the author, Kenneth Brower, describe Curtsinger's adventures with cetaceans, but he also devotes equal attention to details of Curtsinger's personal life. The book does contain many statements about the biology and natural history of whales; these are occasionally in error and are tossed off carelessly as if the facts of whale life are peripheral to the main theme of the book. Brower is obviously fascinated with the issue of what kind of person would devote his life to living on the remote edges of the Earth, constantly struggling for fleeting encounters with marine mammals. Yet Brower never directly acknowledges this hidden agenda of the book and leaves me wondering about his purpose. Does he simply want to extol the virtues of whales as seen through Curtsinger's eyes and camera, or does he want to say something more general about the importance of wildness and mystery in all of our own lives? In spite of this ambiguity, *Wake of the Whale* should interest anyone who wants a beautiful collection of marine mammal photographs accompanied by a description of what it is like to live near these animals.

Peter Tyack

Peter Tyack is a graduate student in animal behaviour at Rockefeller University, New York.

World fisheries

Food from the Sea. By James Nicolson. Pp.205. (Cassell: London, 1979.) £6.50.

THIS book, which is written primarily for the layman and the fisherman, begins with a brief account of aspects of the marine environment which are of importance to fisheries and goes on to consider some of the notable features of fish populations such as reproduction, migration and short- and long-term fluctuations in stocks. This is followed by a chapter which describes some of the main food organisms harvested from the world's oceans. Obviously it is impossible in a book of this size to deal with every important species but in many respects I felt that there was a certain lack of balance in this chapter. For example, the five species of Pacific salmon fished by five nations are only mentioned in passing, whereas the three dogfish of European waters, only one of which has any commercial value, have two paragraphs devoted to them.

In chapters 3 to 7 the author traces the development of the world's fisheries from the earliest times, through the astonishing technological advances of the last hundred years, to the present day. These chapters are well researched and the historical account, laced with many interesting anecdotes, clearly shows that "the history of fishing is a story of human ingenuity and remarkable technological achievement — marred by carelessness and a wanton misuse of some of the richest sources of food in the world".

He then considers the question of overfishing and describes how the development of fleets of ocean-going freezer trawlers in the 1950s and 1960s had a devastating effect on many of the worlds already over-exploited fisheries. In many cases it was the industrial fisheries which suffered most and as soon as one fishery became uneconomic the fleets simply moved on to uncontrolled exploitation of yet another resource. He then deals with the systematic destruction of the north-eastern Atlantic herring fisheries and discussed the conflict between the traditional fishery on the adult stock with the fishery on the young fish on their nursery grounds for reduction to meal and oil. He then traces the history of the scientific basis for fisheries management and the setting up of the various international organisations with reponsibility for conserving the stocks. He is critical of the scientists, the International Council for the Exploration of the Seas and the North-Eastern Atlantic Fisheries Commission for their failure to conserve the herring stocks.

The author is undoubtedly aware of all the problems involved in the management of a stock but it is unfortunate that some space could not have been devoted to considering the differences between growth overfishing and recruitment overfishing. The latter is an important factor in herring and indeed many other pelagic species which grow little in their adult lives, and the failure of the scientists to understand the relationship between recruitment and stock size was a key factor in the collapse of the herring fisheries.

Looking to the future the author considers the role of fish farming as a means of increasing food production from the sea. Throughout the book one gets the impression that, while not denying that fish meal production has a role to play in utilising the resources of the seas, the author would prefer to see a greater emphasis on the use of fish for direct human consumption. It is therefore surprising that he is not critical of fish farming as practised by most of the developed nations in which fish meal is used to produce luxury protein in the form of species such as salmon and trout.

I have no hesitation in recommending this book to anyone with an interest in the fishing industry, especially to anyone who wishes to understand the background to the present problems facing the industry and the controversy involved in the formulation of a common fisheries policy within the European Economic Community.

J.D.M. Gordon

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Sea guides

Marine Life, by David and Jennifer George (Harrap: London; £16) is an extremely well produced book describing marine invertebrates. The centre section of the book consists of 1,300 colour photographs of high quality, while the two end sections describe the various invertebrate phyla down to family and a "representative species". There are a number of line diagrams in these sections of the book. Technical terms are reduced to a minimum and a short glossary is provided. Nevertheless, readers without zoological knowledge might find the book a little daunting. It is not unlike some of the modern gardening books, with a centre section of colour plates and a fairly technical description at either end.

Although the publishers claim it is an encyclopaedia, it could not be used for identification of different groups down to species. Nor could it be considered as "an indispensable work of reference." It is true that scuba diving has enabled biologists and laymen to see animals in their natural state but detailed identification is still done from fixed specimens even if they are "distorted and colourless on the laboratory shelf." The authors seem to take a rather simplistic view of identification, although David George is on the staff of the Natural History Museum. I agree with the Foreword that it is a fine book but it is not a field guide in the sense that it could be put in one's pocket. It is excellent value for £16 and worth a place on the library shelf of any institution, or anybody interested in marine invertebrate zoology, but it should fulfil the role of a colourful adjunct to a systematics course rather than a substitute for the rigours of formal systematics.

Kenneth Gosner's *Field Guide to the Atlantic Seashore* (Houghton Mifflin: Boston; $12.95) would just fit into a capacious pocket. It lists 907 littoral invertebrates and 140 seaweeds from the NW Atlantic from the Bay of Fundy to

Cape Hatteras, although some species extend beyond these limits. The centre section consists of 64 black-and-white and colour plates which are drawings of the species; the two end sections consist of descriptions of the species with notes on seasonal occurrence and where they are to be found. There is no attempt to divide the species within phyla. At the beginning of the book there are introductory chapters on how to use the book, nomenclature, collecting and preserving specimens, labelling, distribution and habits and zonation, and at the end a glossary. Such a book must be of limited use to collectors on British or European seashores, especially when goods books are already available (see *Nature* **264**, 587, 1976).

David Bellamy is well known as a botanist-cum-broadcaster. His book *Half of Paradise* (Cassell: London; £7.95) bears no resemblance to the other two; it is a breezy and well illustrated account of two Joint Services underwater expeditions to the Chagos Archipelago in the Indian Ocean between 1972 to 1975. They appear to have been a combination of a moderately serious scientific enterprise, a Services training exercise and for gathering material for a BBC documentary programme. Results were obtained on the growth of coral reefs, migration and breeding of seabirds and on the flora and fauna of the islands. The expedition charted the islands, dived on wrecks, had brushes with sharks and robber crabs, rats, mosquitoes and sand flies and generally had an eventful time. The book has a Foreword by Prince Charles, who clearly would have enjoyed being a participant. This is a book worth borrowing from the public library if one can 'take' David Bellamy's mildly irritating style of writing. **J.H.S. Blaxter**

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Fish guides

TWO recent guides to British and European fishes could scarcely offer a greater contrast. Judging from their titles and general appearance, L. Cacutt's *British Freshwater Fishes: The Story of their Evolution* (Croom Helm: London, £6.95) is a serious and (one anticipates) authoritative study of the evolution and biogeography of Britain's freshwater fishes, *Fishes: Freshwater and Marine Species* (Chatto and Windus: London; paperback £2.50) second is slighter, being a collection of colour photographs with modest text fitted into matching spaces opposite the picture. One is written by a journalist professionally interested in fishes, the other by an Austrian ichthyologist and translated and adapted by an English zoologist. The first is pretentious, filled with errors and fails in its objective of presenting a coherent account of the fish fauna; the second is as authoritative as its appearance is modest.

Terofal's little book, a *Chatto Nature Guide* contains approximately 120 colour photographs of living fishes, mostly taken in aquaria. The standard of photography is high and the colour reproduction good. Most of the European freshwater fishes are included but the coverage of marine species is less good. Colour photographs of many of the Danubian species have not been reproduced before in English texts and this is a strong point in the book's favour. The text is organised in systematic order, and for each species included there are short, rather telegraphic, accounts under headings of, characteristics, distribution, habits, and diet, although the use of these headings is not consistent throughout. Errors are few; for example, the nine-spined stickleback has been placed in the genus *Pungitius* for several years but is here referred to as *Gasterosteus pungitius*, and the photograph opposite the caption line, *Molva molva*, is of a torsk, *Brosme brosme*. Such minor errors apart, this is an excellent book and should help fill the stocking of many an angler this Christmas — and probably those of quite a few ichthyologists also.

Of Cacutt's book I wish I could say the same. It starts with a typographical error on the fifth page of the prelims with "A fossile pike" a standard which continues more or less throughout. "Spiney-finned Fish" are referred to so often that I had to resort to a dictionary to check that it was not a permitted alternative for spiny.

The author's intention is said to be to present "the broad spectrum of the multi-million year-old story of Britain's freshwater fishes". There are also discussions of fossil fishes and some geology, and potted accounts of continental drift and of the ice ages, of which the last has some relevance to the subject. The major part of the book, however, is a collection of accounts, of British freshwater fishes, species by species, with heavy emphasis on anglers' interest in weights and records, and on behaviour.

The author's familiarity with the subject seem sketchy. On the interesting biogeography of the Irish freshwater fishes we are told on page 60 that the bream, loach and rudd are indigenous to Ireland and then on page 128 that the bream was introduced, while on page 110 the rudd is omitted from another list of indigenous species. In fact, none are indigenous to that island. The author shows a positive genius for getting terms and names wrong, for example, andronomous for anadromous, Roderick Imprey Murchison for R. Impey Murchison, and *Erythrophthalmus scardinius* for *Scardinius erythrophthalmus*. This trait provides a little light relief in places as on page 12 where Darwin's "*Evolution of the Species*" is described as a classic example of a "stodgy" scientific book, "sheer drudgery to read". Alas, Darwin wrote no such book.

This book certainly cannot be recommended, except perhaps as an example of the old saw that a little knowledge is a dangerous thing.

Alwyne Wheeler

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Happiness is helical

TWO recently published books dealing with aspects of the study of molluscs, they are as different as roast beef and candy floss. *Sea Shells: A Naturalist's and Collector's Guide* (Phaidon: Oxford; £6.50), by Peter and Patricia Newell, is very pretty; it has a saccharin sweetness about it and it will appeal to shell novices. But such a diet cannot satisfy for long. Although this book contains many colour pictures by famous invertebrate specialists like Douglas P. Wilson and Heather Angel, the captioning is poor. The information given is sometimes inaccurate (the beautiful nudibranch illustrated on page 23 is not even correct to the nearest family), and the provenance of the specimens illustrated is not uniformly helpful. The text is undistinguished and there is on page 42 a "Key to the most common classes of mollusks" which will confuse any budding shell expert; it should have been left out. *Sea Shells* is a pleasant coffee-table book, no more.

M.P. Kerney and R.A.D. Cameron's authoritative *A Field Guide to the Land Snails of Britain and North-west Europe* (Collins: London; £5.50) in the Collins Field Guide series is the roast beef. It may require more effort to chew and swallow, but the nutritional benefits are substantial. This is the first compendium to deal comprehensively with the land snail shells of the whole of North-west Europe. There are, of course, fairly good guides in existence, dealing with the land molluscs of individual countries, but some of these are out of date, or so massive as to deter even the professional. The present guide describes and illustrates the 279 known species (plus a few stowaways which have turned up in greenhouses) in a way which will satisfy even the most demanding specialist; at the same time it can be used and enjoyed by

those with little or no previous experience.

The land mollusc fauna increases in richness from north to south in the area covered by the guide. Iceland has only 25 or so species, southern Britain about 100 species, and parts of France over 130 species. This kind of faunistic recording is fascinating, and is based upon data accumulated by a vast army of amateur and professional devotees. Outside ornithology, there are few areas of practical biology where the amateur can play a more effective part than malacology, the study of molluscs. The authors give us all the information we need to enable us to join in the fun. There are chapters on the shell, soft parts of the body, life-history, feeding habits, classification, and an excellent introduction to finding, collecting and preserving land snails. Kerney and Cameron will win the gratitude of all those professionals who routinely identify and handle land molluscs, for surely many young malacologists will be recruited through reading this guide. I should like to see a copy in every haversack, along with the usual maps and ornithological field guides. **T.E. Thompson**

T.E. Thompson is a professional malacologist whose most recent appointment was Visiting Professor of Biological Sciences, Florida Institute of Technology.

For the entomologist of the future

THREE recently published popular books on insects differ a good deal in their scope and treatment of the subject. Professor Karl von Frisch, a Nobel Laureate honoured for his work on bee behaviour, is well known to a wider audience of naturalists for his popular work, *The Dancing Bees*, a book which, to my mind, is very much more interesting than the one now under review. *Twelve Little Housemates* (Pergamon: Oxford and New York; hardback £8, paperback £2.50) is a revised and enlarged version of a book on ten groups of insects found in and around houses, and includes accounts of the house-fly, mosquitoes, fleas, lice, bed bugs, clothes moths, cockroaches, ants and aphids. The species are well chosen and their biology is described accurately and simply, in a way understandable by the complete layman or by a child. The book is hardly detailed enough to satisfy the serious amateur naturalist or the budding entomologist, and the rather forced humour of the text and the Victorian air of many illustrations are not likely to win over a sophisticated reader. But von Frisch is mildly discursive and despite some of the naively phrased passages he is able, in his own words, "to show that there is something wonderful about the most detested and despised of creatures".

Dr Carson Ritchie, an archivist and historian by training, believes, in his *Insects: The Creeping Conquerors and Human History* (Elsevier/Nelson: New York; $7.95) that insects have had crucial effects on human history and sets out to describe some of these. For better or worse, the book is largely a collection of historical anecdotes, rather brashly presented and strung together in a loosely chronological sequence, on such subjects as silk production, singing insects, insects as human food, and insects in medicine and art. Entomology is not really one of the author's strong points and the occasional howlers provide additional surprises in a book already packed with stories intended to excite, intrigue, startle, alarm or confound the reader. Entertaining snippets of information abound on every page. Did *you* know that machinery able to produce 318,504,960 yards of silken thread per day had been installed in Derby in 1718? Or that France imported 57 million medicinal leeches in 1823? (By an elastic definition of the Insecta, Dr Ritchie manages to include earthworms, spiders, palolo worms and *Teredo* among his creeping conquerors.) And there are illustrations of all manner of things from Maori tatooing chisels to what I presume are geisha girls entranced by the music of cicadas. But not all readers will be convinced by claims that the silk industry led to the Industrial Revolution or that *Phylloxera* played an important part in the downfall of Napoleon III. Certainly the scholar will regret the more uncritical passages and the almost complete absence of references in support of so many facts.

By contrast, *Insects: An Illustrated Survey of the Most Successful Animals on Earth* (Hamlyn: London and New York; £10), by Brian Selman *et al.*, is a straightforward account, lavishly illustrated with more than 200 colour photographs, line drawings and diagrams. No doubt it will be the coloured illustrations which will fascinate the layman. They certainly convey a most vivid impression of the diversity of insects and, by often portraying things much larger than life, they enable anyone to see insects exactly as the professional entomologist views them through a stereoscopic microscope. The quality of reproduction is generally high and some are superb portraits of handsome subjects with gleaming cuticles, quivering wings, and alert activity suggested by every bristle! The text that accompanies these pictures is written by a group of specialists and deals in turn with the evolution and distribution of insects, their structure, physiology and development, their habitats and modes of life, their impact on man, and their classification. All these are discussed in an authoritative, modern way, and without over-simplification. The result is a book which depicts very successfully the great variety and biological interest of the insects. It can be enjoyed by a 14-year-old who is keen on biology, but it will also serve as a reference book for all but the more specialised undergraduate courses in entomology. Not cheap, but a good Christmas present, especially for the entomologist of the future.

R.G. Davies

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Traditional remedies

THE great majority of the world's population is poor and their medicines are traditional, being mainly plant or animal in origin. These people either care for themselves when ill or use traditional healers. In both cases the sources of their remedies are well known, and children learn from an early age which plants can or cannot be used, and for what purposes. It was realised some time ago that the plants used in these traditional forms of medicine might well repay scientific investigation and there are various projects underway (and no doubt afoot) for doing just that. This is a long way, however, from the current fad for using herbal remedies instead of modern pharmaceutical products. The fashion seems to be confined to the English-speaking world, no doubt because the inhabitants of mainland Europe have never stopped using traditional remedies for a variety of conditions. This may well be due to the fact that there are still peasants in these countries who know and collect the plants. Would that we had a reasonable reservoir of people here who could do the same, but I am afraid this is not the case. This raises a very important point. The books reviewed here are designed for amateurs, not professional botanists and plantsmen. It is essential that either the plants listed should not be harmful or the descriptions so detailed and clear that mistakes should not occur, within the limits of human fallibility. This is especially important when describing members of the Umbelliferae (parsley) family, most of which look alike to the layman and include both very useful herbs and extremely poisonous plants.

Nicholas Culpeper's Complete Herbal and English Physician (Gareth Powell: London; £15) is a facsimile of the 1826 edition but was first published in the seventeenth century, when medicines were

based on plants and folklore. The plants are listed alphabetially under common names, with a description of uses, habitat and time of harvest. Culpeper also gives the sign of the zodiac with which each plant is associated and the effect this has on its use. He was greatly criticised in the past for his interest in astrology — now only of historical interest. He also lists the temperament of the herbs — hot or cold — and the effect this has on the body. There are sections on gathering and drying the herbs, and on the preparation of medicines. The section of cures for various ailments are not all of plant origin; if you rub black snails on a wart and then stick the snail on a blackthorn twig, the wart will go. The archaic English makes this book difficult to read; and although the illustrations are pleasant, its value is historical rather than practical. A good present for devotees of the past.

Maurice Mességué is a practising herbalist, much of whose knowledge is a family inheritance. His book, *Health Secrets of Plants and Herbs*, has been translated from the French (Collins: London; £7.95) and is somewhat flowery in style but down to earth and practical in content: he knows and uses the plants he writes about. He sensibly keeps the list of plants to a hundred readily available ones, enough as he says for the normal needs of a family. He also omits nearly all those with poisonous or irritant properties; and where they are listed, he is careful to say how they should or should not be used. Reading this book has been a pleasure, even if one is left feeling that intestinal worms are rather prevalent in southern France. One matter which will not meet with complete approval in this country is the author's use of garlic practically as a cure-all. The use of garlic plasters for warts and corns (they work within two weeks) beggars the imagination.

Keith Vincent Smith, the author of *The Illustrated Earth Garden Herbal* (Elm Tree Books/Hamish Hamilton: London; £4.95) is an Australian journalist turned self-sufficiency gardener and writer. His book, described as a herbal companion, is an historical account by various ancient and fairly modern writers, of forty herbs, their "virtues" and uses, with recipes. This is an attractive work, of no great practical use, but obviously compiled with knowledge and affection for the subject. There are a few small errors — the genus *Tropaeolum*, for example, is a member of the *Tropaeolaceae* not *Cruciferae*.

The Encyclopaedia of Herbs and Herbalism, edited by Malcolm Stuart, (Orbis: London; £12.50) is disappointing. It is a pleasure to look at, but tries to cover too wide a field — not possible in the space available. There are too many illustrations — not all relevant — and some duplication of information. The quick gallop through the plant kingdom could have been omitted, as could that through the human body. The food recipes too are unnecessary. The main alphabetical list of herbal plants is well done but there is not enough general emphasis on the care needed in identifying and using such plants. This is the most ambitious of the four books under review, but the least successful.

Rosemary Angel

Rosemary Angel is Officer-in-Charge of the Museums Division at the Royal Botanic Gardens, Kew, UK.



Gathering parsley

Taken from *Tacuinum Sanitatis in Medicina* (14th century)

Nature's jewels

YET more to add to the spate of books attempting to satisfy what publishers have found to be a very rewarding money-spinning exercise these days. Fungi have now come of age, and mushroom and toadstool books are now respectable enough to sit on the book-shelves along with those on flowering plants. It is as if the public in all walks of life have suddenly found the lost chord and whether the books deal purely with collecting and looking at fungi or eating them, or some kind of mixture of both people's appetites are apparently insatiable. Hopefully in the future the public will become more discerning as many of those illustrated texts now available are misleading.

It is refreshing that the present publications under review form a considerably better trio. Each is very different in its approach although inevitably they cover much common ground. There is still the need for a good book on mushrooms and toadstools that bridges the gap between the coffee-table approach and the advanced treatise although these present books partially fill that gap.

Geoffrey Kibby's contribution, *Mushrooms and Toadstools: A Field Guide* (Oxford University Press: Oxford; Hardback £7.95, paperback £4.95) consists of fifty pages of introduction, text, the major part of which is a key, and glossary, and two hundred pages of descriptive data accompanying illustrations. The illustrations are all from original water-colours (by Sean Milne) and seem to have been accurately taken from nature; the colour reproduction has been fairly faithful although the species depicted have a somewhat more lustrous appearance than when seen in the field. The abbreviated descriptions are from Kibby's field experience and so have some credibility. Over four hundred larger fungi are depicted, some of which are rare in Britain and are illustrated for the first time in a British field-guide. The key (with novel colour-coding) introduces the reader to the up-to-date classification. Usage by the uninitiated will only show how useful this approach is over more conventional keys.

E. Soothill and A. Fairhurst's *The New Field Guide to Fungi* (Michael Joseph: London; £7.50), is from the illustrative point of view the other side of the coin; the text by Fairhurst being supported by 252 coloured photographs by Soothill. Some of the latter seem to have been rather poorly reproduced, and some of the subjects have been partially burnt out by the flash technique used. Although the author has field experience, the descriptions in the majority of cases are taken from other field guides or monographic treatments, especially the microscopic data; and some unfortunately are incorrectly determined, for example, *Lepiota sistrata* and *Mycena pura*. The format is so very open that a lot of valuable space is wasted, and by using the same size of photograph, whether tree or minute fruiting body, it is difficult to judge size. In order to appeal to a larger audience some microscopic fungi are included as well as a spattering of North American species, for example, *Polyozellus multiplex*. Both groups could have advantageoulsy been left out, as their inclusion, especially the New World species, detracts from the workability of the key which somewhere in its production has gained the wrong numerals (page 24 read 18 for 17; page 26 read 57 for second 37). The introducing text is quite short with an accompanying glossary and poor line-drawings; two indices are included. The

book does not stand up to its dust-jacket statements suggesting that photographs are greatly superior to coloured paintings, as comparison with Kibby's book will soon show.

The Encyclopaedia of Mushrooms, edited by Colin Dickinson and John Lucas (Orbis: London, £9.95), is an interesting mixture of coffee-table style and scientific approach, and contains a mine of information as one might expect from the title. It needs several readings before the full extent of the details, from eating to electron microscopy, and chemistry to ecology, can be appreciated. The text of 280 pages is largely given over to descriptions with an unfortunate mixture of photographs and often appalling coloured illustrations some of which do not even resemble the fungi they are said to depict. The photographs gleaned from various sources are as might be expected of varying quality depending on the photographers and their wide spectrum of techniques. Many of the photographs are of species which have never been seen in a British publication before and so it is refreshing to get away from the same 'old' photographs of fungi, often misidentified, that one sees in the numerous books now available. As in Soothill and Fairhurst the descriptions are short; many are taken from the literature, as again they do not tie-up with the illustrations and therefore the field collections. Over five hundred larger fungi are illustrated with four hundred and thirty by photographs; unfortunately there are some mistakes, for example, *Mycena pura* has become *Laccaria amethystea,* and so on.

The introduction is a good account of the fungi and makes easy reading for the interested student. It is supported by very well chosen illustrations, and the ecological approach of many sections is admirable. Dickinson and Lucas obviously call on their joint teaching experiences, as they have brought together many of those interesting biological phenomena exhibited by fungi with which the layman is unfamiliar. As the dust-cover says, the *Encyclopaedia* is a combination of field guide, natural history encyclopaedia and cookery book. Indeed it is, Dickinson calling on his own culinary interests for the last.

Each book is well bound and on good quality paper; the printing is clear. Some mis-spelling of latin names is unfortunately inevitable and Soothill and Fairhurst's contribution is worst for this — even the dust-cover has one!

Whereas Kibby's contributions could be tucked into a 'poacher's pocket' and taken into the field; Soothill and Fairhurst's book is an awkward size; and Dickinson and Lucas' production was obviously designed for use in the home and library.

Roy Watling

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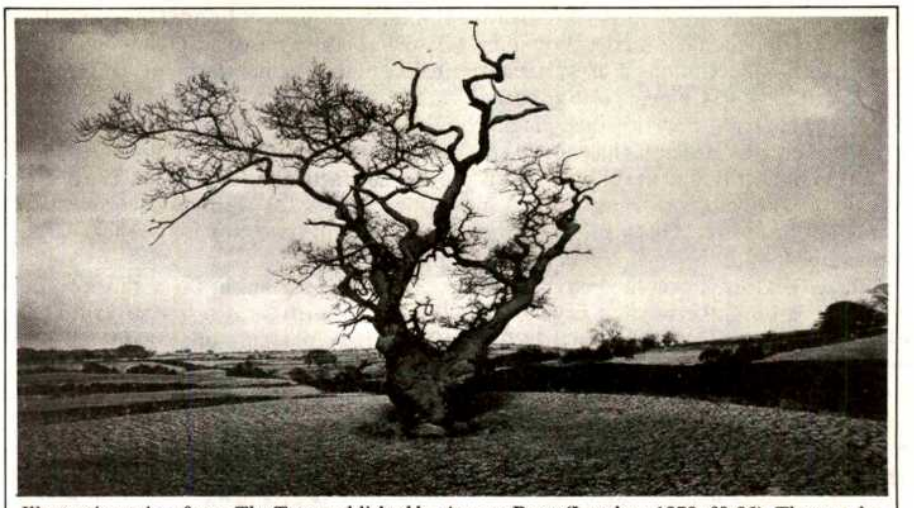


Illustration taken from *The Tree*, published by Aurum Press (London, 1979; £9.95). The text, by novelist John Fowles, accompanies some beautiful photographs by Frank Horvat. Fowles "explains the impact of nature on his own life, and warns urban 'civilized' man of the deeper and more subtle dangers that attend his traditional rejection of the wild.".

Arboreal devastation

After the Elm, edited by B. Clouston and K. Stansfield (Heinemann: London; £7.50) is described as both a requiem for the elm and a guide to the future care and improvement of the countryside. The first chapter, by Brian Clouston, describes the history of the elm in Britain with an emphasis on its role in the landscape. Reference is made to the works of John Evelyn and Gilpin, and there are quotations from Tennyson, Thomas Hood and Herbert Read. My only criticism is that the author persists in describing the wych elm (*Ulmus glabra*) as an introduced species, while as Eddie Kemp makes clear in the next chapter "The Plantsman's Elm", it is unquestionably native. Kemp himself wisely avoids becoming embroiled in controversies over elm taxonomy and presents a straightforward description of the principal types of elm to be found in Britain. Many of the photographs illustrating the trees are, however, disappointingly dull.

Kathy Stansfield's description of "Elm in the Service of Man" overlaps somewhat with the content of the first chapter, but also describes the use of elm wood in the construction of carts, pumps, furniture and boats. Then follow two useful chapters on the biology of Dutch elm disease (D. A. Burdekin) and on the impact that the disease has had on our elm populations (Peter Jones). While appropriate reference is made to methods of disease control, I would have liked to have seen some allusion to the important tree populations which have so far been preserved by control programmes. The Huntingdon elms of Hove park and the magnificent English elm around the Brighton pavilion deserve to be made the object of pilgrimage

The last third of the book is devoted to a discussion on tree planting. There are cogent criticisms of some current fashions — for example, that of planting in field corners — and useful comments on the choice of species. Thus the authors point out that the dark cypresses suitable to Italy create in Britain an effect of "gloomy walks and green-slimed stonework". There is some speculation on the extent to which the current enthusiasm for wood-burning stoves could lead to a renewed interest in coppicing as a system of woodland management, and also a welcome emphasis on the necessity for the adequate maintenance of newly planted trees. In summary, although less visually exciting than Gerald Wilkinson's *Epitaph for the Elm* (Hutchinson: London, 1978; Arrow paperback 1979; £4.95; for review, see *Nature,* **276**, 641; 1978) this is a more satisfactory book. A proportion of the royalties will go to the Tree Council for the National Tree Fund.

Robert lamb's *World without Trees* (Wildwood House: London; £4.95) is an anguished response to the devastation caused by Dutch elm disease, and there are sections on the elm, on the infection process, and on possible control measures. Interleaved with these are other chapters dealing with huge topics such as the destruction of the tropical rain forests, and the world-wide consumption of wood and wood products. Mr Lamb's racy style is often exhilarating and he has made a commendable attempt to describe the complex story of the disease cycle and the host's response in non-technical language. Sometimes, however, his intoxication with words carries him away: "fungal livestock, usually in the form of spores, may now become smeared into the notch by the carrier beetles jockeying for mouth-holes on the sap-rich wood".

More seriously, a lack of authority pervades the whole text. To take one tiny example he describes tyloses as "loops"; presumably because he has only thought of

them in two dimensions. A few pages later he states that the vertical furrows in the bark of elms "serve to prevent water-logging of the trunk by channelling off storm water". One wonders how smooth-barked trees like beech manage to survive at all! He speculates wildly on the subject of plant pathology. For example, he suggests that the ice age may have altered the course of evolution "by way of wholesale disease epidemics long after the climate itself had stabilised". The current deforestation by man is therefore seen as "a speeding-up of a natural process which would happen even if humans did not exist". Although one shares his concern over the way in which the world is using its forest reserve, the chapters on this topic fit incongruously into the framework of the book. I doubt if anyone can read it without feeling both breathless and confused.

John N. Gibbs

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Botanical enthusiasm

SOME books are written to entertain and others to educate. Many books fail to achieve either aim, and a few manage to perform both functions. Naturally, the kind of book which *Nature* selects for review tends to be of the informative type, but even within these one may seek hopefully for a glimmer of aesthetic appeal lurking between scholarly brown covers. The collection of botanical publications reviewed here happily departs from the general run of scientific tomes dissected in these columns and consist largely of books for the amateur, the student or the enthusiast (the three are not necessarily coincident).

In the area of field guides especially, publishers undertake the task of conveying information to a non-technical audience in an accurate yet attractive and pleasing way. Five of the books here may be placed within this group and of them the most novel is *The Audubon Society Field Guide to North American Wildflowers: Eastern Region* by W.A. Niering and N.C. Olmstead (Knopf: New York; $9.95). This book is novel in a number of respects; first in its format which is small, if fat (almost a genuine pocket book), and its flexible plastic binding. It is evidently designed for hard wear in the field. Second it has a thumb tab on the pages of illustrations which indicates the colour and form of the flowers or inflorescences featured on that spread. Illustrations are arranged primarily on grounds of colour and then upon such characters as symmetry, or its lack, in individual flowers and their arrangement approach to the difficult task of leading the user to a group of species which he may compare with the unknown specimen.

There are, however, many criticisms which can be levelled at this approach. It departs from any strictly taxonomic basis for identification. All but the purist would find this forgivable as long as the characters used eventually permit accurate identification. In this case there are certain problems in obtaining this end. The illustrations, which provide the basis for identification, are colour photographs; however good these may be, it is impossible to portray all those features of the plant (growth habit, basal and stem leaves, inflorescence and flower structure, and fruit) which could have been more effectively represented by art work. A second criticism concerns the separation of the text from the illustrations. The text is full, informative and written in an informal style; it also refers to many species which are not illustrated. The general coverage (658 species) is only half that of Peterson and McKenny's *Field Guide to Wildflowers* (Houghton Mifflin: Boston; 1968) which remains the best available field guide for the north-eastern United States.

E.N. Kozloff's book, *Plants and Animals of the Pacific Northwest* (University of Washington Press: Seattle and London; paperback £6.30), undertakes the gargantuan task of providing an introduction to the entire natural biota of an area which approaches 80,000 square miles. Obviously the book cannot attempt a comprehensive coverage and it concentrates upon plants (including some bryophytes, lichens and fungi), vertebrate animals (apart from birds) and some invertebrate groups, such as molluscs. The text descriptions are lucid and contain an enthusiasm and casual detail which tell of the author's evident first-hand experience in the field. It is extensively illustrated by colour photographs which are generally of a higher quality than those in the Audubon guide. The coverage is very selective, but it succeeds in conveying considerable quantities of information in a pleasing and attractive manner. It must be highly valued by naturalists in that part of America.

The Oxford Book of New Zealand Plants (Oxford University Press: Oxford; £32.50) by L.B. Moore and J.B. Irwin also attempts a wide botanical coverage, from diatoms to palms. The format follows the familiar pattern of preceding Oxford guides with facing pages of text and plates. It is a very welcome book in that so little is available in the way of illustrated guides to New Zealand plants; and the artwork is both accurate and pleasing. Representatives are selected from major families of plants found in the Islands and are illustrated in considerable detail, floral sections often being included. It is sad that the bulk of the plates are two-tone as the coloured illustrations are quite superb and the book succeeds in being both entertaining and informative . Although dealing with a flora from the opposite side of the earth, this work should appeal to botanists and horticulturalists in Europe also, for some genera, such as *Acaena* are known as wool aliens and are now established immigrants, whereas others, such as *Hebe* are important decorative genera.

Blandford Press (Poole, UK) have made a brave attempt to fill the great monocot gap and have issued an illustrated guide to *Grasses, Sedges and Rushes in Colour* (£3.95) by M.S. Christiansen. This group of plants is sadly neglected among the current field guides, but I rather doubt whether this particular book will provide what is needed for the uninitiated to identify accurately these tricky plants. C.E. Hubbard's *Grasses* (Pelican, first published 1954) and A.C. Jermy and T.G. Tutin's *British Sedges* (Botanical Society of the British Isles, 1968) are far superior in that they provide keys and in that the illustrations are detailed and accurate. Colour is of little importance for the portrayal of these groups but as distinction often involves details of floral or fruit structure or, in the case of grasses, certain vegetative details, such as the ligule, these need to be clearly visible in the illustrations. In the Blandford paintings one cannot see the hairy ligule of *Sieglingia,* nor the prominent awn in *Holcus mollis* which helps differentiate it from *H. lanatus.* The attempt by the artists to exaggerate the

VOYAGER I

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differences in hair distribution within these two species is not at all successful. Perhaps there is a need for a good field guide to these groups which is less formal and has more popular appeal than the two works mentioned above, but this is not it. When will an intrepid publisher move into bryophytes and lichens, I wonder? The *Oxford Book of Flowerless Plants* (Oxford University Press) has taken a step in this direction, but much remains to be done.

The Audubon Society of the United States has into the coffee-table market with its *Book of Wildflowers* (Harry N. Abrams: New York) by L. Line and W.H. Hodge. This is a large book which combines bigger, better colour photographs (five frontispieces gradually focusing in on a single *Eschscholtzia* bloom 30 cm in diameter), with a blotting paper text, and large print evidently designed for those myopic botanists who get past the frontispieces. As an example of botanical photographic art, the pictures in this book would be very difficult to beat. Every possible photographic technique is used to enhance the glamour of the angiosperm's reproductive structures, which is a perfectly good reason for publishing this book. The text is arranged by habitats, is casual and informal in style, and is reasonably informative. It evidently seeks to convey something of the excitement and appeal of field biology, which is a laudable aim.

Two books which appear to be aiming for the conveyance of information rather than the stimulation of gasps of rapture among their readers are those dealing with the description of flowering plant families. P.H. Davis and J. Cullen have issued a second edition of their key, *Identification of Flowering Plant Families* (Cambridge University Press: hardback £6, paperback £1.95) found wild and cultivated in north-temperate regions — first published by Oliver and Boyd in 1965. Although it is not a subject which any longer receives great prominence in most undergraduate botany courses, classical taxonomy still forms the basis of much field ecology and its neglet is resulting in a generation of students trained in concepts and lacking the skills of field identification, and sometimes even observation. It would be a useful exercise to encourage students to work through a key like this at their leisure, both in natural habitats and in gardens. It should prove a useful supplement to V.H. Heywood's *Flowering Plants of the World* (Oxford University Press, 1978).

R. Headstrom's *Families of Flowering Plants* (Thomas Yoseloff: London; £12.50) lacks the key of Davis and Cullen's book and falls very far short of Heywood's book in its content and presentation. The book contains a discussion of 212 families, including some gymnosperms arranged in alphabetical order of English names, and deals with each family by selecting species of interest and describing their structure and traditional uses. This is not a book for anyone interested in taxonomy, evolution, biogeography, ecology or morphology.

Is it reasonable that one region of botany has been totally ignored by the entire scientific community simply because of its non-existence? This is a question framed by L. Lionni in his book *Parallel Botany* (Wildwood House: London; Paperback £3.95). Why has it not been reported in *Nature* that the flowers of *Anaclea taludensis* vaporise instantly on contact with any object which is not part of their normal ecological environment (see Kamicochi, *Proc. Int. Bot. Congr.* Tsuchimachi, 1974)? Is this indeed a demonstration of Leibschmidt's law that "for every immobility in time there is a corresponding immobility in space"? If you find this kind of question intriguing, then this is the book for you, as a long as you are contented with questions and do not seek answers. A book for the Botany Common Room rather than the library; definitely designed to entertain rather than to inform.

The Story of England's Flora (Kestrel: London; £6.95) by E. Hyams is an attractively produced and lavishly illustrated book. The text leads us on a brief and rather superficial tour of palaeobotany in which the information is reasonably accurate but is so patently second or third hand that it lacks conviction. It proceeds to describe trees, shrubs and herbs (both native and introduced) which have an interest for man. The line drawings are inaccurate (hirsute and crenate *Fagus* leaves), inappropriate (Lombardy poplar in a text piece on the Tertiary) and misleading (a woodland referred to as beech on chalk in which the mature trees are oak and which is almost certainly upon a clay with flints substrate). The book is in part redeemed by some quaint anecdotes concerning the introduction, origin and the breeding of some domstic plants. Another sadly superficial book which deals with a fascinating subject is *Plants that Eat Animals* (Nelson: Nashville and New York) by J.H. Prince. It is a survey of insectivorous plants, their structure, mode of action and physiology. Even though it is intended as a popular book, in the sense that it appeals to the general public, it could have been so greatly improved if the results of modern research had been included. How can one write such a book without including scanning electron micrographs of the glands of *Pinguicula* and without any detailed discussion of the transfer of 'nervous' impulses in plants? Instead we have a simplistic essay illustrated by crude line drawings.

Any review of botany books which attempt to combine education and entertainment would be incomplete without reference to David Bellamy. Two books have emerged on to the market following his *Botanic Man* television series. The book with the same title as the series (Hamlyn: London; £6.50) is a didactic summary of the ideas presented over the television screens. It contains many double-spread colour photographs (a 40 cm diameter bromeliad competes well with the Audubon spectacular in the battle to blow the mind) but Bellamy puts these diversions to far better use by punching home attractively presented information in the intervening pages. He seems to be aiming for the advanced school audience, but how different his approach from Lawson's *Textbook of Botany!* The range of concepts he covers is impressive (from the Periodic Table, through DNA, nutrient cycling at a global and ecosystem level, vegetation zones in relation to climate, plant productivity, even to island biogeographic theory). The text, like David Bellamy himself, is forceful, concise and energetic; it must be read with a South London accent. But above all, this is a living, enthusiastic book which does not avoid or skim lightly over awkward questions; it meets them head on.

A second booklet, *Botanic Action* (Hutchinson: London; hardback £4.95, paperback £3.95) is literally an activity book for children, giving them ideas on how to design simple experiments at home, such as growing seeds in used tea bags to look for mineral deficiencies. Or, how about making paper from stinging netle fibres, or leaf skeletons, or plant based dyes, or dandelion tea? If you have active children interested in their surroundings and if you are not too worried about the state of the carpets, this is just the book to give them for Christmas. Then is the time to judge whether a book can be both informative and entertaining.

Peter D. Moore

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Guide to alpine flowers

The Alpine Flowers of Britain and Europe. By C. Grey-Wilson. Pp.384. (Collins: London, 1979.) Hardback £5.95; paperback £4.50.

THIS is a most useful field guide to the flowers of the hills and mountains of Britain and the continent. It spans from Cape North to the Abruzzi and from Southern Yugoslavia to Ireland. The colour illustrations are from the excellent paintings of Marjorie Blamey. The geographical area covered is about the same as in that most successful book of 1967 by Anthony Huxley *Mountain Flowers* (Blandford: Poole, UK); but the lower altitudinal limit of 1000 metres adopted as a criterion for inclusion, has increased the

number of species illustrated, though most of the additions are really not "alpines". Over the wide range in latitude – from 42° to 71° N –spanned by this book the tree limit descends from some 2300 metres on the Alps to near sea level in Northern Scandinavia. Tree limit is usually taken as the lower boundary of alpine vegetation. It is ecologically much more significant than an arbitrarily fixed altitude, meaningless over such a huge latitudinal span. This is no criticism of the content, only of the title of this book. Anyhow, many of the readers will botanise from foothills to high mountains; for them this field guide caters well, in spite of its somewhat disconcerting title.

There are a number of good features. Text and illustrations are face to face. The description of each species is appropriate and succinct, and it helps to have the diagnostic features in italics. Two keys based on drawings of flower shapes and one, for trees, based on leaf shapes help with preliminary identification. A number of plates give comparative drawings of one or other important diagnostic character in the case of the species of a few difficult genera or families: for instance, calices for the genus *Dianthus*, fruits for some umbellifers, and so on. The illustrated glossary is very clear.

Three minor criticisms: the use of English names for families; the invention of an English name, by translation from the latin binomial, for species which do not have one; and the omission of the author's name after the botanical name. We should not fall into the deplorable habits of American field guides. This guide is dedicated to the Alpine Garden Society which celebrates its Golden Jubilee this year. It will make a most useful companion, as a pocket field flora, to the book *Mountain Flower Holidays in Europe*, just published by that Society. I am sure all members, and those of the sister Scottish Rock Garden Club, will need, and make good use of this guide.

G. Pontecorvo

G. Pontecorvo retired in 1975 as a member of the research staff of the Imperial Cancer Research Fund in London, UK.

Orchid growing

Orchids in Colour. By B. and W. Rittershausen. Pp.192. (Blandford: Poole, Dorset, UK, 1979.) £4,95.

THE attraction of this book by two well-known orchid-growers lies principally in Robin Fletcher's beautiful photographs, the authorship of which, despite the book's title, is not even acknowledged on the jacket. Over one hundred different species or hybrids are illustrated, sufficient to give an idea of the astonishing range of form and colour of these plants, whose increasing popularity is easily understood.

The introductory text comprises general remarks on the Orchidaceae, followed by separate sections dealing with the history and cultivation of the principal groups now grown. This first part suffers from an occasional tendency to become romantic at the expense of botanical accuracy, with such remarks as those with regard to nourishment (which "the orchid has learnt largely to do without"); the "meagre needs" of epiphytes "are totally satisfied by the moisutre of the atmosphere". Fortunately in the separate sections that follow, quite full notes on various aspects of orchit culture are given, particularly as regards temperature and humidity (both of which are critical to successful orchid growing), the use of composts, and the timing of repotting and other cultural practices. Under modern centrally heated conditions, many orchids can be grown in a normal living room, and the suitability of various groups is duly noted. Brief notes on pests and diseases are also included in these sections of the book.

Apart from the breeding of new hybrids, large numbers of which are created every season, modern orchid propagation relies for many groups on "meristemming", the excision and subsequent culture of the growing tip of a shoot; and it would have been useful to include a more detailed description of this practice. There is also virtually no account of the genetics of the extremely interesting and complex nature of multigeneric hybridisation.

Detailed notes (facing each plate) are given on the history and culture of the actual varieties illustrated, and these also deal with related species or hybrids. As a whole, however, the text could do with considerable editing: besides a tendency to be repetitious and thus sometimes confusing, it is at times so ungrammatical as to be obscure and irritating for the reader to a degree that should not occur in a book of this style and authority. One point in its favour is the small format of a volume that one might have expected to see published as a much larger 'coffee-table' book.

Peter Collins

Peter Collins is a freelance science writer who has been studying the orchids of Europe for more than 25 years.

Man's use of dry lands

Dry Lands: Man and Plants. By R. and M. Adams and A. and A. Willens. Pp.152. (Architectural Press: London, 1979.) £12.95.

This volume is written by two landscape architects, a horticulturalist and an agriculturalist, who are concerned to put their expertise to use in the careful management of dryland ecosystems. As a background to this aim the categories of arid land and their special characteristics are represented in some detail. Causes of aridity are discussed, and methods of supplying an index of its severity presented. This is followed by a survey of Northern and Southern Hemisphere desert zones; desert morphology and soils are presented next and desert vegetation types described, including methods of adaptation by plants to arid conditions. This leads on naturally to plant selection for arid regions and the way in which man may manage and modify desert ecosystems. Subsequently, survey and planning methods are expanded, and design and implementation procedures are outlined.

To deal in detail with such an extensive remit would be difficult and in seeking ways to overcome these difficulties the authors have been both inventive and shallow in turn. Overall, the book is attractive in both its presentation and treatment; it follows through from ecological considerations to methods of developing areas agriculturally, for forestry and horticultural purposes, and then to urban landscaping and the development of a coastal site — marina, shops, hotels, airport hotels and gardens. The designs for urban spaces look attractive but the nearest I have experienced to them, in practice, is in Venezuela rather than in the arid zones of Africa or the Middle East. In the course of developing such plans a site survey checklist is presented. This includes hydrological features, geology, topography, soil characteristics, climate, and ecological components. Enough detail is given of the necessity to consider each feature in turn but usually this would not allow a basic understanding of the way in which the various factors operate or how they interact. Where attempts are made to give a basic understanding (as where features of C_3, C_4 and CAM photosynthesis are explained) these can be confused in their presentation and attribute findings to authors who have merely carried along investigations initiated decades previously by other workers.

As an interesting excursion into the application of ecological expertise to planning and land management in difficult situations, this book can be recommended. The work is a useful guide for the ecologist seeking to apply his knowledge. For the planner or landscape architect, seeking the scientific basis for his art as applied to the arid zone, it falls short of a *vade-mecum*.

M.J. Chadwick

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Fascination for fossils

FIVE recently published books on various aspects of prehistoric life and the evolution of the vertebrates are reviewed here. All are intended to help the layman realise the fascination that fossil life might hold for him.

The Hamlyn Nature Guide to Fossils (Hamlyn: London; £1.95), by Richard Moody, begins with a brief introductory section covering geological time, fossilisation and fossil collection, preparation and curation. There is then a simple key to the identification of fossil material down to phylum or class level. The bulk of the book consists of attractively presented colour photographs of some 200 fossils, mostly identified to generic level accompanied by a description and a brief statement of range, both stratigraphical and geographical. Most of the fossils are of common occurrence, though one or two are to be considered rarities; many have world-wide distribution.

The generic identifications are virtually all correct, and the balance of different groups is nicely judged. The various invertebrate fossil groups are treated in turn, followed by a few vertebrates, trace fossils (strangely conodonts and *Serpula* appear under this latter heading), and plants.

My main criticism concerns the photographs. Several are larger than the frame size allows so that parts of the fossil are missing beyond the margins of the plate. Others are presented in rather unconventional orientations. In other cases more than one view is needed and this could have been easily achieved within the frame-size, as the example of *Leptaena* shows. However, apart from a few ill-preserved specimens and a handful of poorly lit ones, the photographs are of high standard and there is never any doubt about what is depicted.

Although there are labelled diagrams to illustrate morphology, I felt sometimes that the descriptions became a little too technical for the amateur fossil collector, to whom the book is directed. He will no doubt welcome it as an attractive and useful addition to his library. The book would fit into a good-sized pocket and could be used in the field.

Fossils (Collins: London; £3.50), by David Dineley, aims to fill a gap which exists in the palaeontological literature; it is thus neither a palaeontology textbook, nor a field guide to fossil identification. The author has produced a book which presents, in a very readable way, the subject of palaeontology to the layman.

After brief introductory sections on sedimentary processes, geological time and evolution of life, there follows a chapter on plants which, in an effort to be comprehensive, perhaps errs too much towards the rarely fossilised non-vascular plants. Treatment of animal fossils follows mode of life, with plankton and nekton being described before benthos. Terrestrial life then follows, leading on naturally to vertebrates and vertebrate evolution.

Having thus covered the major groups of organisms, the author then looks briefly at fossil communities. Techniques of fossil collecting are prefaced by a code for fieldwork and several conservation-minded caveats. The concluding section suggests areas in Britain where fossils may be sought, with further reading for each area discussed. There is a useful list of books for those interested in going into the subject more deeply.

The book is illustrated with numerous line drawings and sixteen black-and-white plates. Two of the latter were badly blurred in my review copy; I suspect this may be a unique fault to my copy but would-be purchasers should perhaps check the plates before buying. There are minor errors in some plate captions.

Professor Dineley's approach, together with his easy-to-read chatty style should win him applause from those who have been (or are about to be) seduced by the fascination of fossils.

Prehistoric Animals and Plants (Hamlyn: London; £2.95), though nominally by Josef Benes, must be considered essentially as a means of displaying reproductions of prehistoric life as seen by Zdenek Burian. After an introductory five pages of text on the origins of life and on invertebrates, there follows some three dozen more pages of text devoted almost exclusively to vertebrates.

After this preamble we are into the bulk of the book, which consists of coloured pictures of animals or faunas as they may have appeared, accompanied by a descriptive text which has been well translated from the original Czechoslovakian. The plates are reproductions of original paintings by Zdenek Burian and are a tribute to his skill in convincingly reconstructing the animals in their environments.

Although this book is up to date in some respects (it contains, for instance a Precambrian reconstruction based on the Ediacara fauna), in other cases it suffers from being behind the times — as for instance in the pictures of dinosaurs which are based on the "giant lizard" concept of dinosaur physiology. *Brachiosaurus,* for example, is relegated to a watery life, as it has been so often in the past. It is to be regretted that the book is so heavily vertebrate oriented particularly because the few reconstructions of invertebrates are so successful.

The standard of the pictures varies, but it is not clear whether this is entirely due to faulty reproduction; some colours in a few pictures are rather garish, a few images appear to be out of focus, and some pictures were originally painted on a rather coarse canvas to the detriment, sometimes, of detail in the reproduction. The work must represent the output of the artist over a considerable period so that differences in style are not unexpected.

It is not clear to whom this book is directed, but at the remarkable price of £2.95 for a hardback book with over 300 pages and more than 130 colour pictures, it should find a ready market in anyone interested in realistic reconstructions of extinct vertebrates.

The Evolution of the Mammals (Peter Lowe: London; £4.50), by L.B. Halstead, begins with a definition of what constitutes a mammal, and then explores briefly and succinctly geological time, evolution and the fossil record, the origin of life, and then goes into more detail on vertebrate evolution. Thus by the time that mammals are first discussed in detail, the reader should be fully aware of their relationship to other vertebrate groups and their range through geological time.

The Mesozoic mammals are dealt with rather sketchily, but there is a fuller treatment of Cainozoic mammals, initially system by system, in which differences between successive faunas are spelt out clearly in the text and by superb coloured illustrations; some of these illustrate different genera of particular mammalian groups and others, reconstructions of mammalian faunas of different ages in their supposed environments. The pictures are very successfully executed and the considerable impact of this large-format book owes much to them.

There are sections on the evolution of man, on the different faunas to be found in the main continental areas and even predictions on what the future holds for the mammalian class.

The whole book is clearly written, interestingly presented, and succeeds in telling the history of mammals through geological time in a most absorbing way. Although the key words for the British Library Catalogue are listed as "Mammals — Evolution — Juvenile literature", I venture to suggest that most parents who buy this book for their offspring will find it fascinating reading for themselves.

The subtitle of *Archosauria,* by J.C. McLoughlin (Allen Lane: London; £4.50) — *A New Look at the Old Dinosaur* — also appears in large print on the dust cover and will no doubt cause confusion with Alan Charig's new book *A New Look at the Dinosaurs* (Heinemann: London, 1979). However, in content John McLoughlin's book is much closer to Adrian Desmond's *The Hot-Blooded Dinosaurs* (Blond and Briggs: London, 1975).

A zoologist by profession (as are many vertebrate palaeontologists), the author is entirely sold on the idea that dinosaurs were endothermic (warm-blooded) animals, and the book considers the Archosaurs as a class of vertebrates (following the line first proposed by Bakker and Galton).

Each group of archosaurs is discussed and their functional morphology in the

light of an endothermic physiology re-interpreted. The text is well written, though the British publishers have apparently reprinted the American edition, as American spellings persist. The author has provided his own illustrations and succeeded remarkably in selling his argument by managing to give an intelligent and alert expression to the faces of the long-thought slothful dinosaurs. The arguments are put over clearly and persuasively, but the author too rarely considers alternative viewpoints to his own. I found his attitude to classical dinosaur reconstructions somewhat patronising, and felt that the criticisms of earlier workers were not entirely justified. Despite his biased view, the author has produced a book which is certainly stimulating, very readable and attractively presented, and which will appeal to anyone with an interest in dinosaurs, whatever their background.

John C.W. Cope

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In the hands of the rockhounds

MINERALOGY is increasingly in the hands of the rockhounds. Short courses in gemology and the lapidary arts are over-subscribed and will hopefully lead on to a much wider public interest in the earth sciences in general. Sadly, one consequence is that well-known mineral localities, and some which once were near-secret, are picked over and almost barren. Disused workings are being closed for safety reasons and recent legislation all but bans collectors from active mines. For all except the mundane, many collectors are forced to buy imported specimens and professional collecting has become highly profitable. It is not unknown for *bona fide* scientific collectors or the casual amateur to be literally driven off outcrops by well equipped and frenzied dealers or their suppliers. In this context, the publication of three new books aimed at the amateur can be received with some misgivings. Not one of them pays any attention to the pressure on sites nor to conservation practices. That being said, professional geologists should attempt to encourage wider interest and activity in this field as a route to popularising earth sciences. But to what extent do the new books contribute to this aim?

The Hamlyn Nature Guide to Minerals by Andrew Clark (Hamlyn: London and New York; £1.95) is a thin book, in all respects. it claims to be a practical identification guide but its brief introduction to mineral properties is too dry for the beginner. Its 436 colour photographs are pretty enough, but the brief mineral descriptions are not arranged in a suitable format for easy identification. The order is the conventional composition/atomic structure classification, which is no guide to minerals' immediate appearance. No information about localities is given but there are very brief notes on geological setting, which will certainly not aid the non-geologist to find good specimens. Perhaps this is a book for the pure conservationist.

The Audubon Society Field Guide to North American Rocks and Minerals by Charles W. Chesterman (Knopf; New York; $9.95) is in another class. It contains 794 magnificent colour photographs and is virtually a self-contained introduction to geology through its more aesthetic products. The book begins with a simple but comprehensive account of the diagostic properties of minerals. The ordering of photographs does not match that in the identification tables or the extended mineral by mineral descriptions — the photographs being arranged by colour and form, the tables by colour and hardness. But this is only a minor irritation, albeit unecessary. The quality of specimens was a little daunting for this casual collector and perhaps for even more determined rockhounds. This reflects the use of tiny specimens or micromounts — forced on modern collectors by the self-induced dearth of 'mantle piece' specimens. Common rocks are well treated and sufficient space has been devoted to introducing the elements of the science, as well as the hobby, to make this book a notable contribution in the field and excellent value.

Rock and Mineral Collecting in Britain by Peter R. Rodgers (Faber and Faber: London, hardback £6.95, paperback £3.50) is a good text for absolute beginners and relates mineral occurrences to geological setting quite adequately. However, the simplification into igneous, sedimentary and metamorphic classes of deposits can be misleading at this elementary level, and arguably a better approach would have been regional descriptions of famous mineral sites. The localities listed seems impressive, but the reader soon finds those mentioned in the text very elusive. The most useful items are the mineral catalogue and the list of localities with grid references. There are glaring omissions among the localities and the list would have been better organised region by region with accompanying maps. Several famous British minerals, noteably mimetite, harmotome, torbanite and alstonite are in-explicably absent. The beginner will find this an encouraging little volume, but a little extra documentation might have found a ready demand among more experienced collectors.

Stephen A. Drury

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How to grow your own rubies

IF you want an excuse not to give your wife an opal for Christmas, you will find one in D. Elwell's book: *Man-Made Gemstones* (Ellis: Chichester, UK; Wiley & Halstead: New York; £15), both natural and synthetic opals are porous, and serious cracking can occur if they are immersed in warm soapy water.

Far better to give her a ruby — "Growing rubies by the flame fusion process in the garage is possible, but not recommended. We will therefore consider flux growth" . . . Dr Elwell goes on to give detailed instructions, but briefly you take a platinum crucible, mix lead fluoride with the oxides of lead, boron, aluminium and chromium, add a pinch of lanthanum oxide to improve crystal shape, put in the oven at 250°C for 24 hours, allow to cool slowly, and extract your crystals using nitric acid.

Unfortunately, your home-grown rubies, though real, will be "synthetic". This is an important qualification. Synthetic gemstones are identical, chemically and crystallographically speaking, to the real thing; but they are usually distinguishable from, and therefore less valuable than, natural gemstones. 'Imitation' or 'simulated' gems on the other hand resemble the real thing but are actually a different material; thus yttrium aluminium garnet (YAG) and cubic zirconia are both used as diamond simulants.

Opals do have their own fascination, as Elwell explains, even if you cannot yet grow your own. They are unique among gemstones in being an ordered array of silica crystallites, whose spacing leads to diffraction of visible light and hence to a captivating play of colours as the gem is moved. Most other gemstones are genuine crystals — an ordered array of atoms — and are transparent, whereas opal is opaque.

I certainly enjoyed reading Dr Elwell's book. Although ruby is the only crystal he gives a recipe for, he also describes the synthesis of sapphire, spinel, emerald, diamond and its simulants, and many other precious and semiprecious stones.

Gemology (Wiley-Interscience: New York and Chichester, UK; £13.50) by the

Professor Emeritus of Minerology at Harvard (C.S. Hurlbut) and the Curator Emeritus of Minerology at the Smithonion Institution (G.S. Switzer) is a self-confessed "textbook" aimed at gemology courses as well as at jewellers and collectors. As such it is less readable tha *Man-Made Gemstones*; but it is much better as a work of reference — as well as being bigger (in number of pages and page size) and cheaper.

The book covers origin and occurrence of gemstones, crystal chemistry and crystllography, physical and optical properties of gems, gemological instruments, synthesis of gems, imitation gems, cutting and polishing. The longest chapter, "Descriptive Gemology", tabulates all the important features and characteristics of the common gemstones (and some uncommon ones) with particular emphasis on how to tell whether the gem is natural, synthetic or imitation. There are also twelve full-page colour plates of superb quality.

These books are complementary, rather than being in direct competition; and both would make suitable presents for your favourite lapidary or jeweller, or for anyone with a scientific interest in gems and crystals. But if you can afford only one of them, *Gemology* is the bettery buy.

John Walker

John Walker was a Research Fellow at the Universities of Reading and Paris, studying the optical properties of diamond. He is currently a microprocessor consultant.

Everything you wanted to know about cancer but were afraid to ask

DISEASE has always haunted the human imagination. Leprosy, the black Death and other horrors have in turn stalked the misty frontiers of the collective unconscious. As the lethal epidemic diseases recede into history the archetypal phobias of Western Society are shifting their attention to cancer. One of civilisation's most awful self-inflicted wounds, cancer has become, like death itself, a taboo. In the third world the peasants flee to the hills when cholera strikes; in our affluent society there is nowhere to run to avoid what the late John Wayne called the big C. In the belief that fear can be abolished by knowledge (or rather, information) two recent books have been launched — from different directions and aimed at different audiences.

The Cancer Reference Book (Paddington: New York and London; £4.95), by P.M. Levitt and E.S. Gurabrick, is written by two American Professors of English using information provided by a small team of established cancer specialists. It is deliberately written in simple English, avoiding all technical language or complicated concepts. The style is light with quotations from Dylan Thomas, Robert Lowell *et al*, and it is rigorously honest. There is no obfuscation, no undue optimism. If a treatment does not work, the book says so. Reassurance is offered only when justified. It provides brief descriptions of the various forms of cancer and provides lists of questions and answers. The questions are real — the sort of questions asked by patients and their relatives. The information provided is strictly limited to topics of immediate relevance to patients with malignant disease and to the uninformed general public. Symptoms, treatments, prognosis and terminal care are well covered. The long section on widespread cancer and terminal care is very well presented and pays an envious tribute to the British hospice movement. Some aspects of the dialectic are (at present) peculiar to North America. Unproven treatment methods such as laetrile and the orgone box receive a lot of critical attention and furthermore the authors recommend that all men over 50 should have a rectal examination once a year. They also deal with "cancer insurance" in some detail, as in the USA 2 million such policies are sold every year. The cost of treatment for breast cancer can be as high as $100,000 per patient.

Assuming that the reader knows absolutely nothing, the book contains simple line drawings most of which are probably helpful. However, one picture of the mouth labelled "the mouth" seemed just a little superfluous. However, this volume cannot really be judged by doctors. It is for the general public and for patients. To assess its value I have just completed a small uncontrolled trial by inviting questions from patients, relatives and friends. Each and every question was answered, and answered well, although finding them by referring to the index was a little frustrating at times. *The Cancer Reference Book* contains, as advertised in its subtitle "Direct and clear answers to everyone's questions". It must therefore be judged a considerable success. Its honesty will I suspect upset some doctors more than their patients. It could make life a little difficult for those, who, with euphemisms and woolly reassurance, use deception as a form of therapy. *The Cancer Reference Book* could be a blow for patient's lib.

Cancer: the Outlaw Cell is an edited collection, by R.E. La Fond, of articles originally published in *Chemistry* and now dressed up and issued by the American Chemical Society (Washington DC; $15). It is a distinctly upmarket product. Written originally for chemists (no, not pharmacists) it now seems to be addressed to what I can only describe as "a BBC2 audience". Expensively produced and beautifully illustrated its approach is conventional but slick (witness the title). Clinically it is uninformative and contains little or nothing about what cancer is or what it does to people. It is essentially a basic primer in some current trends in cancer research, very basic in places. An introduction to cell chemistry, for example, takes less than a page and a half. There are, however, some excellent general essays. Dr Armin C. Braun's chapter on cancer as a problem in development will fascinate anyone with an O level in biology. There are also comprehensive chapters on chemical carcinogens, oncogenic viruses, the cell surface and the inevitable immunology.

A simple but concise essay on cancer chemotherapy by the Drs Burchenal highlights a central issue in contemporary cancer research. The logical approach to finding new drugs, they say, would be to find differences between normal and malignant cells and then exploit the difference. However, as this approach has been tried in the past and failed, it has been replaced almost totally by empirical drug screening, looking for anti-proliferative agents, agents which by definition, are bound to be toxic. To illustrate the current obsession with "multi-modality therapy" they provide an unfortunate analogy. The body, they say, is like a lawn, normal cells are grass and cancer cells are weeds. Surgery is therefore the equivalent of hand weeding, radiotherapy is a flamegun and chemotherapy is (or should be) a selective weedkiller. Stimulation of the immune response is the equivalent of stimulating the grass to choke the weeks. As they put it so succinctly, immunotherapy is fertiliser.

It is an optimistic book, the authors of each chapter arguing strongly for their own line of research and its potential value. It is an excellent bit of public relations for American cancer research; no room here for self-doubt, for the currently fashionable pessimism. As an introduction to cancer for the scientifically literate layman it could make a fascinating read.

G.A. Currie

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• In the review of *Malignant Neglect* (see *Nature*, **282**, 166; 1979), the first sentence of the second paragraph should have read: "A normal metabolite, ammonia, is called (page 105) a "toxic organic vapor"."

Man's place in Nature

The Ancient Science of Geomancy: Man in Harmony with the Earth. By Nigel Pennick. Pp. 180. (Thames and Hudson: New York and London, 1979.) $16,95; £5,95.

FOR those who believe that a proper understanding of our world can come only through the application of the scientific method — by observation, hypothesis, prediction and test, leading to verification or otherwise — this book will read as mystical nonsense. For those who believe that there are other, less rigorous ways to the truth, who prefer a mystical to an archaeological or historical approach to the past and who are also attracted to the idea of a return to a technologically simple and harmonious human culture, it may well have considerable appeal. This conclusion is quickly reached by reading the Introduction in which the author's basic assumptions are clearly stated they are vast, irrational and of a nature that precludes almost any kind of constructive criticism.

> "The practice of geomancy, which may roughly be defined as the science of putting human habitats and activities into harmony with the visible and invisible world around us, was at one time universal, and vestiges of it remain in the landscape, architecture, ritual and folklore of almost all countries in the world. This remarkable series of correspondences between different cultures has been held to be evidence for a former world civilisation . . . These archetypal patterns . . . have produced the worldwide occurrence of outward form and inner purpose found in geomancy. Hence the seeking of cosmic power points on the surface of the Earth, special places where the mind can expand into new levels of consciousness . . ."

If one accepts these basic assumptions — a better phrase would be "this basic faith" — then all the rest follows. The book elaborates on the theme, describing a wide range of curious facts and phenomena as if their mere recounting is enough to prove the author's case. The Thoms' work on standing stones is inevitably brought in, as if this particular example of the refutation of the old simplistic idea of the steady progress of man from savagery to modern urban civilisation also proved the existence of a world-wide science in antiquity. Ley lines appear, as does dowsing; and holy wells, holy hills and geometrically designed buildings are thought of as places where the natural energies of the Earth were identified and tapped in ancient times. Proofs of the power of these energies — as of all aspects of geomancy — are invariably sought in unattributed and unverifiable reports of "evidence", as of an allegedly sacrificed animal removed from its burial place, of disasters following and ceasing with the return of the corpse to its tomb. Adverse criticism is exorcised in advance by allegations of bias and prejudice among professional scientists and scholars. The mind reels at the task — fortunately unnecessary — of analysing and assessing this mass of diverse and incoherent material. Probably it is best simply to regard the work as a compendium of bizarre and interesting phenomena and tales.

One general point, however, is worth making. The thread running through the book is that in early times (what archaeologists call the Palaeolithic, or hunting and food-gathering period) man lived in complete harmony with nature and that this harmony was later disrupted with the invention of agriculture. In a typical phrase, full of apparent meaning but defying close analysis, "settled life necessitated the enclosure of space, a stopping of the free flow of the spirit of the earth . . .". All signs of ancient skills and wisdom are interpreted in terms of early Man's knowledge and utilisation of the Earth forces assumed to exist behind such harmony. However, this "harmony" can also be interpreted quite unmystically in terms of the natural (that is, unconscious) selection of technologically primitive societies so that those survived which were best adapted to their environments. No alien spacemen, antediluvian Atlantean civilisations or practitioners of a world-wide geomantic science are needed — but a thorough knowledge of the available archaeological and historical evidence is.

This makes the point forcibly that, in the 120 years which have elapsed since the publication of *The Origin of Species*, the application of the theories of evolution and natural selection to the development of man and his societies has made too little headway. The second half of the Darwinian revolution is thus still incomplete and, as a result, a host of beliefs and dogmas based on assumptions about the special nature of *Homo sapiens*, and on his independences, of natural laws, continues to flourish. Until, in T.H. Huxley's phrase, "Man's place in nature" is properly and widely understood and accepted there will still be massive markets, even among educated people, for books of this kind in which the effects of natural selection are viewed anthropocentrically and attributed to primeval wisdom, or are explained by supernatural or extraterrestrial agencies.

Euan W. MacKie

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Glimpses of volcanoes

Volcanoes. By M.B. Lambert. Pp.64. (David and Charles: London and Newton Abbot, UK; University of Washington Press: Seattle, 1978.) £3.95; $10.95.

IT is amazing how little the author has managed to cram into this slim volume. The book runs to 64 pages, over half of which are devoted to pictures and diagrams, and it is divided into 10 principal sections, each of which consists of a few pages of text, some relevant pictures, a surprising amount of blank space and a vaguely volcanic snatch of verse. The sections cover fairly conventional topics such as "Major volcanic landforms", "Products of explosion", "Volcanoes in time and space", and so on. Unfortunately, the contents of the sections do not match up to their worthy titles. The section on "Volcanoes in time and space", for example, runs to only a few hundred words, about half of which are devoted to Canada.

It is difficult to see quite what kind of a book the author was aiming at. One could make a strong case for the publication of a good coffee-table book on volcanoes, but this one does not come anywhere near it, as although many of the photographs used are good, they are not numerous enough, not glossy enough, and not in colour. The book is also emphatically *not* a "simply yet thorough introduction to volcanoes", as the dust-jacket claims, but is instead a patchy, misleading digest of information on a subject which is inherently very popular among lay-readers.

Such readers are likely to fare badly from Lambert's book. What, for example, is the uninformed reader going to make of the 400 words on page 9, which includes a discussion of thermal gradients, upper mantle composition, partial melting and fractional crystallization? Not much, I suspect. The best that can be said about the text is that it is at least accurate for the most part, being derived from respectable sources to whom Lambert makes due acknowledgement. In many cases, however, Lambert's use of these sources is curiously ill considered. He quotes, for example, Macdonald's figure of 1,800,000 km^2 for the extent of the so-called 'Thuelan Plateau'. It is now generally accepted that it was never anything like this size, the various components having been widely separated by seafloor spreading. Macdonald even goes so far as to suggest this possibility, but Lambert ignores it.

Peter Francis

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Frozen Earth

The World of Ice: A Natural History of the Frozen Regions. Pp. 120. By B.S. John. (Orbis: London, 1979.) £5.95.

THIS attractively produced book would look well on any coffee-table and should arouse interest in the World's ice in all its varied mainifestations. Brian John has written a stimulating and, for the most part, basically sound account of the ice-covered or frozen regions of the Earth that assumes little acquaintance on the part of the reader with technical terms, whether geographical, glaciological or biological. (A glossary is included.) It is therefore a book for the interested layman, the general reader, and only superficially for the student, the scientist or the naturalist. It is illustrated by about 100 superb colour plates, some covering a page or double-page spread; the layout is spacious, the quality of production high and the price very reasonable.

The text is clearly written but, inevitably, compressed and simplified, so that the unwary or a reader unfamiliar with the subject can be misled at times. Just to select a few examples, we are told that the World's climate has for most of the time been very much colder than at present (page 10), ignoring the long spans of the geological past when it has actually been much warmer, with no polar ice; that the strandflat was "eroded by the sea" (page 19); and that a mechanism of great importance to the movement of the Antarctic ice-sheet is basal sliding (page 47), giving the unfortunate impression that it is peculiar to the Antarctic. The caption to a photo of northern Skye (page 32) describes it as "glacial terrain" but the main processes actually depicted are connected with mass movement; sheet-jointing in Yosemite (page 61) is attributed to temperature variations (no mention of pressure release); and the caption to a photo of Bodmin tors (page 60) gives the impression that frost was the major formative process. In all such statements there may well be a grain of truth, but brevity and sometimes an unfortunate choice of words can easily convey quite the wrong idea. The opening page (page 7) is distinctly journalistic ("We are all basically afraid of extreme cold and darkness"); and the inhabitants of the Alps or Scandinavia must be amused to read that "Something akin to panic is induced... every time there is a heavy snowfall".

There are seven chapters: the ice planet; ice environments; glaciers; frozen ground; ice afloat; plants, animals and ice; and Man in the World of ice. Some important subjects are given litte mention: avalanches are dismissed in about 70 words; the section on plants is comparatively thin; and in the chapter on Man, there is virtually no mention of the economic importance and utilisation of the permafrost areas of Siberia, Alaska and Arctic Canada. But the overall impression of the book is undoubtedly attractive and it should find a ready market on the bookstalls.

C. Embleton

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Immediacy and surprise

ASTRONOMERS are extremely fortunate members of the scientific community because they have an efficient public relations network. It may be an unwitting PR network — astronomers don't pay for it anyway — but the media do seem to be on their side. Two things are greatly in the subject's favour. First is the immediacy. The results and images from a spaceprobe passing Jupiter, for example, are transmitted and processed with great speed. The time lag between observation and announcement is short. Second is the element of surprise. Time and again the astronomers find things they did not expect. Jupiter and its satellites again furnish two examples. It would have been a brave astronomer who firmly predicted the existence of active volcanoes on Io or a ring around the planet. Pioneer found both. New things seem to crop up wherever the astronomer looks and nothing seemingly interests the media more than novelty coupled with those looks of amazement on the faces of scientists when confronted with something unexpected. Two consequences result: the number of people interested in the subject continues to increase, and astronomy books seem to date quickly, providing ample room and justification for new works and editions.

This year, as in previous years, I am confronted with a large pile of new books and I am again trying to answer the question "Which book would you, young astronomer, old astronomer, amateur or professional, like to find in your stocking on Christmas morning?" The choice is enormous and the answer is far from easy. One which I am sure will go down very well is *The Guinness Book of Astronomy Facts and Feats* (Guinness Superlatives: Enfield, UK; £6.95), compiled by Patrick Moore. It is the quiz fanatics' dream, a fund of answers to questions you probably would not have asked yourself in the first place. Every dip I made into the book produced something of interest and humour. Who would have thought that Le Monnier observed Uranus eight times in four weeks and failed to identify it as a planet, or that one of his observations was later found scrawled on a paper bag which had once contained hair perfume; that the longest living sunspot group lasted for 200 days; that Halley's comet was excommunicated in 1456; that the smallest known galaxy is only 1,000 light years across; that a piece of the Barwell meteorite was found nestling in a vase of artificial flowers on the windowsill of a house in the village? The book is full of them; you can imagine the advertisement, "astound your friends", "impress your colleagues" — but it's all good fun and excellent value. The facts and feats are listed in sections, a random few being Sun, Moon, Jupiter, double stars and galaxies, so they are easily accessible. Eighty-seven pages are taken up by a star catalogue which struck me as being somewhat out of place. The star maps in this catalogue were too small, had no right ascension or declination grid and the bright stars in each constellation were joined in a most unusual, idiosyncratic fashion. And I am sure that 10^{-8} cm as the definition of an Angström unit is easier to comprehend than "one hundred millionth part of a centimetre." Anyway, these are all minor quibbles. I know few astronomers who would not be delighted to receive a copy.

The same can be said of the next book, *Space Art* compiled and written by Ron Miller (Starlog Magazine, O'Quinn Studios: New York; paperback $7.95, slipcase delux $12). We are treated to a multitude of colour plates of astronomical and space science subjects realised by a host of artists. The book continually underlines the usefulness of space art. These paintings synthesise scientific knowledge and data into a visual and concrete form. Flesh is put on to the bones of numbers, equations and theoretical predicitions. Whenever a new phenomenon or discovery comes to light it is the space artist who asks the question, "What would it actually look like to the human eye if you could stand there and see it?". Ron Miller traces the evolution of this art-form from its infancy in 1865, when *From the Earth to the Moon* and *A Trip Around the Moon* by Jules Verne, contained illustrations created by the artist painting for the first time strictly according to scientific fact, through to the present day, where we benefit greatly from such organisations as the NASA Fine Arts Programme. NASA decided that, despite filmed documentation of all their projects, art would give their work a special emotional impact and significance. Miller goes as far as to propose that it was the space art in the popular illustrated magazines and books of the early 1950s that helped rally the taxpayers behind the space programme. Well of course, this is debatable, but the worth of the present book is beyond dispute. It is excellent value for money, a continual feast for the eye and the imagination and an ideal gift.

Let us now turn to two books for the observer. The Mittons, Jacqueline and Simon, have produced a *Star Atlas*



(Jonathan Cape: London; Crown: New York; £1.10). This is a very handy, reasonably priced, 32-page, large-format paperback, half of which is devoted to a description of such topics as mapping the skies, the constellations, star names, magnitudes and the way the atlas is to be used; the remainder contains the maps, 2 for the north and south celestial poles, declinations from 90° to 60°, and 12 for the equatorial region of the celestial sphere, each of these being 5 hr across, allowing some overlap. My first response to this book was to compare it with *Norton's Star Atlas* (Gall and Inglis: Edinburgh; £4.50). Norton's book goes down to magnitude 6.35 and contains slightly more stars than the Mittons'. Norton represents his stars by filled black dots, scaled in half-magnitude steps. The Mittons represent stars by spidery, computer-drawn asterisks, the star magnitudes being indicated to within 0.1 mag by the size of the printed star image (you need a good engraved scale and a magnifying glass to make use of this).

There is considerably more text in Norton than in Mitton, although people who can get most from the atlas do not really need the text. There is a more comprehensive list of doubles, variables, clusters and nebulae in Norton. I prefer Norton's dots to the Mittons' asterisks, although I prefer the Mittons' price to Norton's. However, if I was buying an amateur star atlas I would still save up for Norton.

The second book for the amateur observer is *The Messier Album* by J.H. Mallas and E.Kreimer (Sky Publishing Corporation: Cambridge, Massachusetts; Cambridge University Press: Cambridge; $9.50; £6.95) and I recommend this most heartily. Owen Gingerich provides an historical introduction to Messier and the catalogue is reproduced in facsimile, but the real kernel of the book is the descriptions, drawings and photographs of each object. We are not confronted here with 100-inch Schmidt photographs but with the realistic capabilities of amateur astronomers. Mallas has a 4-inch refractor and he draws and describes exactly what can be seen of each Messier object using this instrument. The photographs were obtained by Kreimer using a 12.5-inch *f*/7 reflector, with the guiding head, low-temperature, plate camera at the newtonian focus. About eight pictures could be obtained during a 4-hour observing session. The book ends with a series of colour photographs and again the technique used to obtain each of these is given in detail. Everyone with a telescope should have this book.

The remainder of my Christmas books have a bias either towards the fundamentals of astronomy or towards space science. *Basic Astronomy* by Patrick Moore (Ian Henry: Hornchurch, UK; £3.95) is a modest, small-format second edition of the 1969 original. The production quality reminds me of the books published around the time of the second World War, most of the photographic reproductions being worse than one would see in the average newspaper. The figures suffer from *déja vu*, all seemingly having been plucked from some ancient tome. The text is vintage Patrick Moore and I like it. You cannot help being infected by his enthusiasm. He writes about what he knows; you could drive a cart and horses through the omissions. At the same extremely elimentary level is *Discovering Astronomy* by Jacqueline and Simon Mitton, Institute of Astronomy, University of Cambridge (Longman: Harlow, UK; Prentice-Hall: Englewood Cliffs, New Jersey; £4.75) . I quote the address of the authors in full because this is given in large letters on the cover. We are also told on the cover that "For the inquiring young person there is no first-step introduction to the 'new' astronomy written with the authority commanded by authors at Cambridge Univeristy's Institute of Astronomy." Why should we care where the authors come from as long as they have written a good book? I am sure that an author could write a 'first step' into astronomy without going anywhere near a university, never mind Cambridge. Inside we find a superbly illustrated 90-page introduction to astronomy, ideal for the infant and junior school room. Adults wishing to take more than one step into astronomy should start elsewhere. However, I would not specifically recommend *Foundations of Astronomy: From Big Bang to Black Holes* (David and Charles: Newton Abbot, UK; £5.95) by Richard Knox. It is billed as a new and radically different introduction to astronomy for the lay or student reader. I found it to be neither new nor radical but simply a reasonably competent,

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methodical plod through the usual topics.

As time is short and the number of simple astronomy books enormous, it is important that the new reader, young or old, does not waste time or become discouraged by making the wrong choice for his introductory text. The Americans come to our rescue here. I have previously praised *University Astronomy* (Saunders) by J.M. Pasachoff and M.L. Kutner, and *New Horizons in Astronomy* (Freeman) by J.C. Brandt and S.P. Maran, and I have no hesitation in adding the works of William J. Kaufmann III to this short list. He has produced a trilogy of books for the general reader. I have before me *Planets and Moons* and *Stars and Nebulae* (Freeman: San Francisco and London; hardback £8 and £8.20, paperback £3.95 and £4.10). The line drawings are superb, clear, uncluttered, eminently understandable and a perfect example to every writer of how it should be done. The illustrations are beautifully reproduced and relevant to the text. Not for Kaufmann the collecting together of any handful of NASA prints just to add spice. The text hammers home the important points, the new ideas, the problems and the next steps. It is unfair to single out one topic from the rest but I will. Kaufmann's exposition of the birth, life and death of stars is masterly. This is how introductory books should be written. There is no need to plod through mediocrity when the best is so easily available.

Saturn and Beyond (Lothrop, Lee and Shepard; New York; $7.95) by Isaac Asimov, is a rather pedantic stroll through the features of Saturn, Uranus, Neptune and Pluto. We are told that this is Dr Asimov's 199th book. I got the impression that Dr Asimov has slightly run out of steam on this one. Why are rings common? How many theories of ring origin are there? Why do these planets have similar spin periods? Why haven't we detected planets beyond Pluto? Why were Neptune and Pluto so difficult to discover? Why are the spin axes at different inclinations, and why are the interior structures of these planets dissimilar? We learn too little about these problems in this book; we are treated to too many tables, too little explanation, too few illustrations. Even the US Naval Observatory's photograph of Pluto and Charon, arguably one of the most exciting outer planetary photographs of the decade, is missing. Never mind, Kaufmann's book has it.

The Astronomy Data Book by J. Hedley Robinson and James Muirden (David and Charles: Newton Abbot, UK; £6.95) is a revised but little improved version of the 1972 original. I must not be too harsh with this book because it contains many useful tables listing astronomical objects and their characteristics. It is also aimed much more towards the amateur astronomer than C.W. Allen's classic *Astrophysical Quantities* (Athlone Press: University of London). However, I cannot help but feel that it could be made much better. The Glossary is very poor, and throughout the book the definitions need tightening up, with more quantitative assertions as opposed to qualitative ones. The few figures the book contains leave much to be desired and many more figures are needed. The knots in which the authors tie themselves as they try to describe the Moon's orbit, collars on Venus, zodiacal light and sidereal time, to mention but a few, have to be read to be believed. The new index is a great help.

Finally, let us have a look at four space books. Closest to us in a physical sense is A.T. Lawton's *A Window in the Sky* (David and Charles: Newton Abbot, UK; £6.50). Lawton lifts the astronomical observer through the Earth's atmosphere and has written a most readable account of the achievements, possibilities and problems of observing from satellites. He discusses the astronomical objects that benefit most from this type of observation and the instrumentation required. The main emphasis is on the non-visual parts of the electromagnetic radiation spectrum, but even so I was rather surprised about the omission of a chapter on the Space Telescope.

Passage to Space: The Shuttle Transportation System (William Morrow; New York: $6.95) by Charles Coombs, and *The Space Shuttle: Its Story and How to Make a Flying Paper Model* (Lothrop, Lee and Shepard; New York; $6.50) by Frank Ross Jr, are both short, profusely illustrated, simple accounts of the USA's next step in space exploration and are both aimed at the younger reader. There is very little to choose between these books; they are both excellent introductions to the subject. However, if I must choose, I would buy Frank Ross's (incidentally, the model flies beautifully).

We end this review with a flight of fantasy. *The Handbook for Space Pioneers* (Westbridge Books/David and Charles: Newton Abbot, UK; Grosset and Dunlap: New York; £5.95) by L. Stephen Wolfe and Roy L. Wysack, discusses the activities of a hypothetical organisation known as GAIL, the Galactic Association of Intelligent Life. Starships have roamed the neighbouring regions of our Galaxy searching for habitable planets. The book introduces pioneers who fancy leaving Earth to the physical characteristics and state of development of eight new worlds and it also provides a brief review of the starships that will be used to transport these emigrating Earth citizens. This book should appeal to all space flight enthusiasts and science fiction fans. The fun the authors had in producing it sparkles from every page.

David W. Hughes

David W. Hughes is Lecturer in Astronomy and Physics at the University of Sheffield, UK.



Illustration taken from *The End of the Game*, reviewed on page 640 of this issue.

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Pipette tip. Variable Volumetrics now introduce a new yellow pipette tip to cover the range 5–250 μl. The tip is called the Video Bonus Tip and features a very small hole. This helps to expel the last drop and thus to ensure accuracy.
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A range of the Variable Volumetrics pipette tips

Inhalation chamber. Wahmann. A proven and tested 200-l inhalation chamber custom engineered for research and toxicity testing of a large variety of substances is now available from Wahmann manufacturing. The chamber, of stainless steel construction, can accommodate removable animal caging and racking for a variety of animal species and is thoroughly factory tested to maintain a negative pressure for a specified period. The chamber features sampling ports and particulate measuring instrumentation to meet any specific requirements. In addition, blowers, filters, illumination and viewing ports as desired are readily available. A free brochure with further information is now available.
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Microwave oven system. Photovolt Corporation introduces a new microwave oven system for fast and accurate moisture measurements of food, dairy and pharmaceutical products. Called the Apollo Mark XII, the system gives results in 3 min. An LED readout displays either per cent moisture or per cent solids. To operate the Apollo, samples are placed directly in the oven and the built-in electronic balance automatically tares, weighs and sets itself to 100%. When the sample is dry the oven automatically turns off. Operational instructions are shown on the LED display. Microprocessor control makes the whole sequence automatic.
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Balances with an automatic weighing feature. Arbor. A new series of balances from Arbor Laboratories, using advanced microprocessor technology features the new Auto Weight/Auto Tare. This provides immediate weighing results without the 'flutter' associated with conventional weighing systems. There is also a new auto precision feature, with which the user can simply dial in the desired level of precision required.
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Sealing film. Sealonfilm is now available from Alpha Laboratories. This is a tough sealing plastic film which will adhere to pottery, glass or metal and give an airtight, leakproof seal which can be used in the temperatue range -70 to 55 °C. It has high resistance to ethanol, methanol, concentrated acids and alkalis and is also resistant to organic solvents.
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Twin strainers. Royles. A new brochure describes the Royles Type 'G' twin strainer detailing materials of construction, component identification chart, weights, dimensions and a flow rate/pressure drop chart as an indication to size and capacity. Based on the provision of two strainer cages in a single body combined with suitable valve gear mechanism, a twin strainer allows one cage to be removed and cleaned while the other remains in operation. The standard sizes are from 3″ to 18″ (larger sizes also available as specials) and are capable of handling from 110 to 3,800 gallons per min given a 2 p.s.i. pressure drop through the unit.
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Light scattering photometer. Chromatix. A new Application Note describing the use of the KMX-6 low angle laser light scattering photometer to determine molecular weights and molecular weight distributions of low molecular weight polystyrenes is now available from Chromatix.
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Electronic top-loading balances. Brinkmann Instruments announce the introduction of two new electronic top-loading balances offering much greater capacities at accuracies of 400 g to 0.001 g or 3,000 g to 0.01 g. These new balances are microprocessor equipped and are available with optional BCD-output for connection to printers, calculators, and other data handling equipment.
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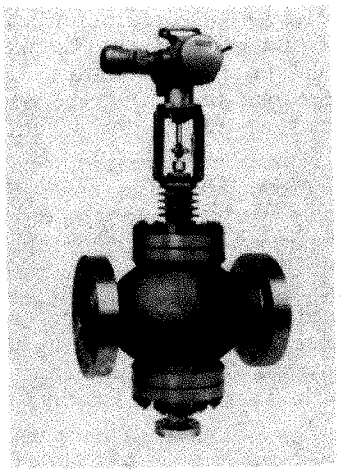
Heated gas cell. Specac. Gas cell 5700 from Specac has been designed so that spectroscopic studies can be made of gases and vapour at temperatures above ambient up to 250°C. This enables vapour and gas spectra to be obtained of substances that are in either the solid or liquid phase at room temperature. This cell has a 120-W heater around the body which enables the temperature of the sample to be raised to 250°C. A vacuum stop cock is fitted which is made of stainless steel and operates over the whole working temperature range of the cell. An injection septum is fitted for the introduction of gas or liquid sample. The seals in the cell are made either from silicone rubber or PTFE. A copper-constantan thermocouple is supplied with the cell and passes through a vacuum tight seal into the gas cavity so that the temperature of the gas sample can be monitored.
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The Specac gas cell 5700

Mechanical booster pump. Edwards. The EH4200 booster vacuum pump is now available from Edwards. Suitably backed, it has a pumping speed of 3,400 $m^3 h^{-1}$ and ultimate pressure of 1.3×10^{-5} mbar. Pumps in the Edwards EH range, which now cover capacities from that of the EH4200 down to 400 $m^3 h^{-1}$, have higher speeds and compression ratios than other pumps of similar dimensions. Hydrokinetic drive allows them to work at optimum speed and with minimum power demand throughout the entire pressure range, and whatever the gas load.
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Steam-transforming valve. Arca-Regler. The steam-transforming valves from Arca-Regler have the capacity to reduce and at the same time desuperheat. The design includes a pressure-balanced plug, which is also the explanation for the relatively small actuator. The four-flange body, taken from a standard valve programme, has at the bottom flange a connection facility for the cooling water line. The mixing of the reduced steam with the cooling water takes place in an area of heavy turbulences directly behind the seat of the reducing plug. The valve shown in the figure has been constructed for a conventional power station, where superheated steam of 106 bar and 535°C (±10°C) is reduced and mixed with feed water of 130°C.
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The Arca-Regler steam-transforming valve

Dual piston pump for HPLC. Schoeffel. A dual piston, constant volume pump for HPLC has been introduced by the Schoeffel Instrument Division of Kratos. The pump includes a full complement of the necessary valving and flow controls for immediate use with any LC detector and injection valve. It provides precise control of flow rate from 0.1 to 9.9 ml min^{-1} with an accuracy of better than 1% (using methanol or water at 2 ml min^{-1}). Operating pressure of the pump may be as high as 6,000 p.s.i. A detailed description of the HPLC pump and its corresponding accessories and system components is available from the company.
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Convection ovens. Boekel. Double wall electric gravity convection ovens are now available from Boekel. These ovens are used for sterilising, drying, conditioning, pre-heating, baking, evaporating, annealing, dehydrating, and curing in any laboratory. The double wall construction includes an all-metal chamber and an outside sheathing of Marinite XL. All walls and doors are insulated with 3 inches of rock wool. Approximately 80% of radiant heat up to 460°C is reflected; destructive heat scaling up to 660°C is resisted.
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Block digestion systems catalogue. Techne announces the publication of a new 4-page catalogue describing all component and accessories of its new block digestion systems. These units are designed for sample preparation before analysis of grain, food products, soil, water pollutants and chemicals.
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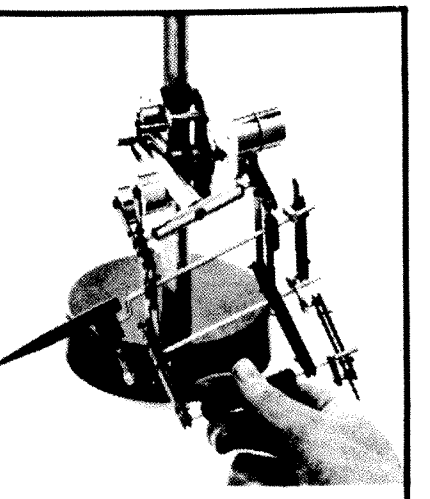
1979-80 Biochemicals catalogue. Miles. The 1979-80 edition of the Miles Biochemicals Catalog is now available, free of charge, from Miles Research Products. The catalogue's 150 pages include listings of over 2,000 product sizes and types in 10 different product groups. There are detailed descriptions of 980 products under the headings of immunochemicals, lectins, blood proteins, electrophoresis apparatus and reagents, polypeptides, nucleic acids, recombinant reagents, enzymes and companion reagents. To help locate specific products, listings are cross-indexed under major subject and individual product headings. Suggested applications, with relevant cross references, are also provided for most products.
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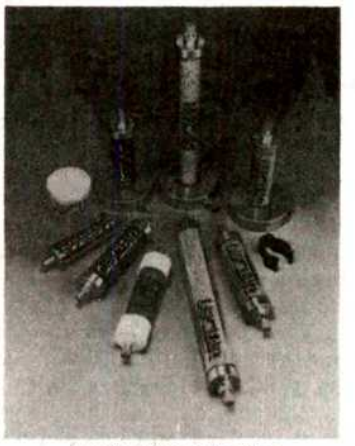
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LabClear filters and purifiers

Automatic oxygen monitor. Neotronics. Automatic oxygen monitoring with Neotronics Otox 90s linked to nitrogen control valves permits operators dispensing radioisotopes to concentrate on the job in hand and not worry about oxygen levels inside the manipulation cabinets they are using. The Otox 90 is designed for both field and fixed installation use and operates either from mains supply or its own integral Ni-Cd battery. A resolution in 0.1 per cent steps over 0–35 per cent oxygen is provided. Audible alarm points may be set either side of a desired level to give immediate warning of excessive deviations.
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Spill Control Pillows. Lab Safety Supply. Spill Control Pillows from Lab Safety Supply can be used to absorb spills of hazardous chemicals, biologicals or radioactive materials. Pillows are applied directly to the spill, absorbing most spills within 30 s. No clean-up of neutralisers or absorbents is necessary — the Pillow completely contains the spill-saturated absorbent for safe, convenient and fast disposal.
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Balances. Weighdata. Three new models in the Thor range manufactured by Weighdata are now available. The DK4/13 is a dual range model accurate to either 1 g in 12 kg or, using the same balance, 0.1 g in 4 kg. The M4000 is a single balance in a new compact casing accurate to 0.1 g in 4 kg. Both models are designed for laboratories and small chemical process plants for both weighing and component counting. The third new design is the DT2500, a single balance accurate to 0.01 g in 2,500 g for general laboratory use.
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Electromechanical and electronic components catalogue. Radiatron Components has introduced a new version of its popular shortform catalogue. As its predecessor, it is intended to provide a brief visual summary of the Company's capability in precision electromechanical and electronic components and systems. Products covered in the 'Shortform' include interconnection devices, instrument cases, boxes and housings, collet fixing knobs, relays, miniature cooling fans, a wide selection of switches, surge arrestors, and a host of panel components. The 'Shortform' can be obtained on request and comes with a special reply-paid card to facilitate further response and consequent assistance.
Circle No. 148 on Reader Enquiry Card.

Multichannel recorders. Gulton. Features of the fixed range and plug-in signal conditioner chart recorders from Gulton include super shock resistance (15 g for 11 ms), dry thermal writing, recording in any orientation, plug-in signal conditioners for measurement versatility, 125 Hz frequency response, slim profile for true portability, rugged steel encased coaxial styli, pushbutton chart speed selection, right hand event marker and remote chart on/off.
Circle No. 149 on Reader Enquiry Card.

Brochure on films. Kodak. A new brochure entitled "KODAK Films for Fluorography and Spot Film Radiography" (M3-149) is now available from Eastman Kodak Company. The brochure offers complete product descriptions of Kodak PF film, a medium-contrast, high-speed orthochromatic film, and Kodak PFC and Kodak X-Omat GR films, high-contrast, medium-speed orthochromatic films. The latter film is available to meet the needs of spot-filming cameras that require 10 cm × 10 cm square-format films. Charts, sensitometric comparisons, and processing recommendations are included to assist diagnosticians in film selection and quality control procedures. Also listed are film features, codes and catalogue numbers and film sizes.
Circle No. 150 on Reader Enquiry Card.

Recent scientific and technical books

Biology

AGUAYO, Albert J., and KARPATI, George (ed.). In association with BRAY, Garth M., EISEN, Andrew A., LIVETT, Bruce G., and RASMINSKY, Michael. Current Topics in Nerve and Muscle Research. (Selected Papers of the Symposia held at the IVth International Congress on Neuromuscular Diseases, Montreal, Canada, September 17-21, 1978.) Pp.ix + 327. ISBN-0-444-90057-8. (Amsterdam and Oxford: Excerpta Medica, 1979.) Dfl. 120; $58.50.

ALPER, Tikvah. Cellular Radiobiology. Pp.viii + 320. ISBN-0-521-22411-X. (Cambraidge, London and New York: Cambridge University Press, 1979.) Hardcover £18; Paperback £5.95.

ARMSTRONG, Frank Bradley, and BENNETT, Thomas Peter. Pp.x + 491. ISBN-0-19-502406-0. (New York and Oxford: Oxford University Press, 1979.) £14 net.

ASHBURNER, M., and WRIGHT, T.R.F. (ed.). The Genetics and Biology of Drosophila, Vol. 2c. Pp. lxxxix + 617. 1978. £32.

ATASSI, M.Z. (ed.). Immunochemistry of Proteins, Vol. 3. Pp.xviii + 321. ISBN-0-306-40131-2 (v.3). (New York and London: Plenum Press, 1979.) np.

BARBIER, Michel. Introduction to Chemical Ecology. Translated by M. Ferenczi. Pp.xiii + 128. ISBN-0-582-44378-4. (London and New York: Longman, 1979.) £3.95 net.

BARNES, Ervin H. Atlas and Manual of Plant Pathology. Pp.xviii + 325. ISBN-0-306-40168-1. (New York and London: Plenum Press, 1979.) np.

BATES, William H. The Bates Method for Better Eyesight Without Glasses. Revised edition. Pp.188. ISBN-0-583-12920-X. (London: Mayflower, Granada Publishing, 1979.) £1.25.

BELL, P.A., and BORTHWICK, N.M. (ed.)s Glucocorticoid Action and Leukaemia. (Proceedings of the Seventh Tenovus Workshop, Cardiff, April 1978.) Pp.xii + 217. ISBN-0-900663-07-3. (Cardiff: Alpha Omega Publishing, Ltd., 1979.) np.

BENACERRAF, Baruj, and UNANUE, Emil R. Textbook of Immunology. Pp.vii + 298. (Baltimore, MD.: The Williams and Wilkins Company, 1979.) $10.95.

BERRIOS-ORTIZ, Angel, and SELANDER, Richard B. Skeletal Musculature in Larval Phases of the Beetle *Epicauta segmenta* (Coleoptera, Meloidae). (Series Entomologica, Vol. 16.) Pp.v + 35 + figures 1-184). ISBN-90-6193-126-6. (The Hague, Boston, Mass. and London:M Dr. W. Junk B.V., Publishers, 1979.) Dutch guilders 50; US$26.35.

BOETHEL, David J. and EIKENBARY Raymond D. (ed.). Pest Management Programs for Deciduous Tree Fruits and Nuts. Ppxi + 256 ISBN 0-306-40178-9 (New York: Plenum Press, 1979). $29.50

BONADONNA, G., MATHE, G., and SALMON, S.E. (ed.). Adjuvant Therapies and Markers of Post-Surgical Minimal Residual Therapies. (Recent Results in Cancer Research, Vol. 67.) Pp.xviii + 150. ISBN-3-540-09291-9. (Berlin and New York: Springer-Verlag, 1979.) DM 48; $26.40.

BONADONNA, G., MATHE, G., and SALMON (ed.). Adjuvant Therapies and Markers of Post-Surgical Minimal Residual Disease II: Adjuvant Therapies of the Various Primary Tumors. (Recent Results in Cancer Research Vo. 68.) Pp.xi + 465. ISBN-3-540-09360-5. (Berlin and New York: Springer-Verlag, 1979.) DM 98; $53.90.

BRADLEY, J.D., and FLETCHER, D.S. A Recorder's Log Book or Label List of British Butterflies and Moths. Pp.136. ISBN-0-902068-08-3. (Plaistow, London: Curwen Books, 1979.) np.

BRECCIA, A., RIMONDI, C. and ADAMS, G.E. (ed.). Radiosensitizers of Hypoxic Cells. Ppix + 258 ISBN 0-444-80124-3. (Netherlands: Elsevier/North-Holland Biomedical Press, 1979) US $44.00/Dfl. 90.00.

BRODA, E. The Evolution of the Bioenergetic Processes. Revised reprint. Pp.x + 231. ISBN 0-08-018275-5 (Hardcover) ISBN-0-08-022651-5 (Flexicover) (Oxford: Pergamon Press Ltd, 1979). Hard £14.00 Flexi £6.50.

BRUSSARD, Peter F. (ed.). Ecological Genetics: The Interface. With contributions by R.W. A.llard et al. (Proceedings in Life Sciences.) Pp. x + 247. ISBN-3-540-90378-X. (Berlin and New York: Springer-Verlag, 1978.) DM46; $25.30.

BUSCH, Harrid (ed.). The Cell Nucleus. Vol. 7: Chromatin, Part D. Pp.xxix + 601. ISBN-0-12-147607-3 (v.7). (New York and London: Academic Press, 1979.) $49.50.

CHARALAMBOUS, George (ed.). Liquid Chromatographic Analysis of Food and Beverages, Vol. 2. (Proceedings of a Symposium held in Honolulu, Hawaii, April 1-6, 1979.) Pp.xii + 237-563. ISBN-0-12-169002-4 (v.2). (New York and London: Academic Press, 1979.) $25.

CHARALAMBOUS, George (ed.). Liquid Chromatographic Analysis of Food and Beverages, Vol. 1. (Proceedings of a Symposium held in Honolulu, Hawaii, April 1-6, 1979.) Pp.xiii + 236. ISBN-0-12-169001-6 (vol. 1.) (New York and London: Academic Press, 1979.) £10.40.

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CLARK, Wallace H., GOLDMAN, Leonard I., and MASTRANGELO, Michael J. (ed.). Human Malignant Melanoma. (Clinical Onsology Monographs.) Pp.xii + 509. ISBN-0-8089-110-4. (New York and London: Grune and Stratton, 1979. Distributed in UK by Academic Press, London.) $39.50.

COHEN, Georges N., and HOLZER, Helmut (ed.). Limited Proteolysis in Microorganisms: Biological Function; Use in Protein Structural and Functional Studies. (Conference held at National Institutes of Health, Bethesda, MD.) Pp.vii + 255. (DHEW Publication No. (NUH) 79-1591). (Bethesda, MD.: National Institutes of Health, 1979.) np.

CROOKS, J., and STEVENSON, I.H. (ed.). Drugs and the Elderly: Perspectivew in Geriatric Clinical Pharmacology. Pp.viii + 304. ISBN-0-333-23800-1. (London and Basingstoke: The Macmillan Press, Ltd., 1979.) £15. net.

DAVIS, W., and ROSENFELD, C. (ed.). Carconogenic Risks: Strategies for Intervention. (Proceedings of a Symposium organized by IARC and the French National Institute of Health and Medical Research, held at the International Agency for Research on Cancer, Lyon, France, 30 November-2nd December 1977.) (IARC Scientific Publication No. 24.) Pp.xix + 280. ISBN-92-832-1125-1. (Lyon: International Agency for Research on Cancer, 1979. Distributed by WHO, Geneva; London: HMSO.) Sw.fr.50; $30.

DICK, Heather M., and KISSMEYER-NIELSEN, F. (ed.). Histocompatibility Techniques. Pp.viii + 212. ISBN-0-444-80132-4. (Amsterdam, New York and Oxford: Elsevier/North-Holland Biomedical Press, 1979.) Dfl. 90; $44.

CACUTT, LEN. British Freshwater Fishes: The Story of Their Evolution. Pp.202 + 25 photographs. ISBN-0-85664-320-3. (London: Croom Helm, Ltd., 1979.) £6.95.

CAMPBELL, Keith O. Food for the Future: How Agriculture Can Meet the Challenge. Pp.x + 178. ISBN-0-8032-0965-7. (Lincoln and London: University of Nebraska Press, 1979.) £8.75.

CARRUTHERS, Malcolm, and MELLETT, Peter (ed.). The Coming Age of Psychosomatics. (Proceedings of the Twenty-first Annual Conference of the Society for Psychosomatic Research, held at the Royal College of Physicians, St. Andrew's Place, Regent's Park, London, NW1, 21st and 22nd November 1977.) Pp.viii + 227-383. ISBN-0-08-023736. (Oxford and New York: Pergamon Press, 1979.) £11.

CARTER, Stephen K., CROOKE, Stanley T. (ed.). Assisted by ALDER, Nancy A. Mitomycin C: Current Status and New Developments. Pp.xiii + 254. ISBN-0-12-161560-X. (New York and London Academic Press, 1979.) $20.

CARTHY, J.D. Revised by HOWSE P.E. The Study of Behaviour Second Edition. Pp.v + 68 ISBN 0-7131-2749X. (London: Edward Arnold Ltd, 1979). £1.90.

CECCARELLI, Bruno, and CLEMENTI, Francesco (ed.). Neurotoxins: Tolls in Neurobiology. (Advances in Cytopharmacology, Vol. 3.) Pp.xviii + 461. ISBN-0-89004-303-5. (New York: Raven Press, 1979.) $39.50.

CELIS, J.E., and SMITH, J.D. (ed.). Nonsence Mutations and tRNA Suppressors. Pp.x + 349. ISBN-0-12-164550-9. (London and New York: Academic Press, 1979.) £16; $33.25.

CHANCE, Britton, MARCUS, Rudolph A., DeVAULT Don Charles, SCHRIEFFER J. Robert, FRAUENFELDER Hans, and SUTIN Norman (ed.). Tunneling in Biological Systems. Pp.xviii + 758. ISBN 0-12-167860-1. (New York: Academic Press Inc. 1979). $42.00.

CHAPMAN, R.F., and BERNAYS, E.A. (ed.). Insect and Host Plant. (Proceedings of the 4th International Symposium, Fulmer Grange, Slough, England, 1978.) (*Entomol. exper. appl.* 25, Nr. 3.) Pp.x + 566. (Amsterdam: Dutch Entomological Society, Plantage Middenlaan 64, 64, 1978) Dfl. 90.

DICKSON James G. CONNER, Richard N., FLEET, Robert R., KROLL, James C., and JACKSON, Jerome A. (ed.). The role of Insectivorous Birds in Forest Ecosystems. (Proceedings of a Symposium held July 13 and 14, 1978, in Nacogdoches, Texas.) Pp.xiii + 381. ISNM-0-12-215350-2. (New York and London: Academic Press, 1979.) $24.

DeLISI Charles and BLUMENTHAL Robert. (ed.). Physical Chemical Aspects of cell Surface Events in Cellular Regulatoin. Vol. 4. Pp.x + 393. ISBN-0-444-00311-8 (Vol). ISBN-0-444-41607-2 (series). (New York Elsevier, North Holland Inc. 1979). $45.00

DeLUCA, H.F. Vitamin D: Metabolism and Function. Pp.viii + 80. ISBN-3-540-09182-3. (Berlin and New York: Springer-Verlag, 1979.) DM29; $16.

DNA: Replication and Recombination. (Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIII, Parts 1 and 2. Part 1: PLp.xxiii + 1-654. Part 2: Pp.xiii + 655-1387. ISBN-0-87969-042-9. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1979.) $120.

DOYLE, G.A., MARTIN, R.D. (ed.). The Study of Prosimian Behavior. Pp.xvii + 696. ISBN-0-12-222150-8. (New York and London: Academic Press, 1979.) £32.15.

DROST-HANSEN, James S. (ed.). Cell-Associated Water. (Proceedings of a Workshop on Cell-Associated Water, held in Boston, Mass., September 1976.) Pp.vii + 440. ISBN-0-12-222250-4. (New York and London: Academic Press, 1979.) $22.

DYER, A.F. The Experimental Biology of Ferns. (Experimental Botany: An International Series of Monographs, Vol. 14.) Pp.xviii + 657. ISBN-0-12-226350-2. (London and New York: Academic Press, 1979.) £37.50; $79.

ERDOS, E.G. (ed.). Bradykinin, Kallidin and Kallikrein, Supplement. Contributors: K.D. Bkoola, *et al* (Handbook of Experimental Pharmacology, Vol. XXV Supplement. Continuation of Handbuch der Experimentellen Pharmakologie.) Pp.xxii + 842. ISBN-3-5450-09356-7. (Berlin and New York: Springer-Verlag, 1979.) DM288; $158.40.

EXTON-SMITH A.N. and OVERSTALL, P.W. Geriatrics — Guidelines in Medicine Volume 1. Pp.vi + 344 ISBN-0-85200-213-0. (Lancashire: MTP Press Limited, 1979). £9.95.

FALKNER, Frank, and TANNER, J.M. (ed.). Human Growth. Vol. 3: Neurobiology and Nutrition. Pp.xvii + 606. ISBN-0-7020-0733-1. (London: Baillière Tindall, 1979.) £24.75.

FAUCI, Anthony S., and BALLIEUX, Rudy (ed.). Antibody Production in Man: *In Vitro* Synthesis and Clinical Implications. (Proceedings of hte Catherijne Conference on Immunology held in Utrecht, Holland, on March 15-18, 1978.) Pp.xiv + 398. ISBN-0-12-249950-6. (New York and London: Academic Press, $18.

FEINBRUN-DOTHAN, Naomi. Flora Palaestina, Part 3. Text: Ericaceae to Compositae. Pp. 481. ISBN-965-208-000-4. Plates. Pp. 757 + xxxiii. ISBN-965-208-003-9. (Jerusalem: The Israel Academy of Sciences and Humanlies, 1978.) $55; IL500.

FISHMAN, William H., and BUSCH, Harris (ed.). Oncodevelopmental Antigens. (Methids in Cancer Research, Vol. 18.) Pp.xxi + 409, ISBN-0-12-147678-2. (New York and London: Academic Press, subsidiary of Harcourt Brace Jovanovich, Publishers, 1979.) $31.

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GANTI, Tibor. A Theory of Biochemical Supersystems and Its Application to Problems of Natural and Artificial Biogenesis. (Studia Biologica Hungarica, No. 17.) Pp. 136. ISBN-963-05-1719-1. (Budapest: Akademiai Kiado, 1979.) np.

GARATTINI, Silvio, GOLDIN, A., HAWKING, F., and KOPIN, I.J. (ed.). SCHNITZER, R.J. (consulting editor). Advances in Pharmacology apf Chemotherapy, Vol. 16. Pp.x + 302. ISBN-0-12-032916-6. (New York and London: Academic Press, $31; £2015.

GERSHENSON, S.M. Principles of Modern Genetics. Pp.502. In Russian. (Kiev: Naukova Dumka, 1979.) np.

GHOSE, T.K., FIECHTER, A., and BLAKEBOROUGH, N. (ed.). Advances in Biochemical Engineering. Volume 12. Pp.vi + 253. ISBN-3-540-09262-5 ISBN-0-387-09262-5 (Berlin: Springer-Verlag, 1979) DM98. US$53.90.

GOTTSCHALK, Gerhard. Bacterial Metabolism. (Springer Series in Microbiology.) Pp.xi + 281. ISBN-3-540-90208-9. (Berlin and New York: Springer-Verlag, 1979.) DM43; $23.70.

GROSJEAN, Daniel (ed.). Nitrogenous Air Pollutants: chemical and Biological Implications. Pp.x + 349. ISBN-0-250-40294-7. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., 1979. Distributed in UK by John Wiley and Sons, Ltd., Chichester.) £18.25.

GUDZINOWICZ, Benjamin J., and GUDZINOWICZ, Michael J. Analysis of Drugs and Metabolites by Gas Chromatography-Mass Spectometry. Vol. 6: Cardiovascular, Antuhypertensive, Hypoglycemia and Thyroid-Related Agents. Pp.x + 446. ISBNO-8247-6757-8. (New York and Basel: Marcel Dekker, Inc., 1979.) SFr.106.

GUNSALUS, I.V. (editor-in-chief). The Bacteria: a Treatise on Structure and Function. Vol. 7: Mechanisms of Adaptation. Edited by J.R. Sokatch and L. Nicholas Ornston. Pp.xx + 593. (New York and London: Academic Press, 1979.) $45.

HABERLAND, Gert L., and HAMBERG, Ulla (ed.). Current Concepts in Kinin Research. (Proceedings of the Satellite Symposium of the 7th International Congress of Pharmacology, Paris, 22 July 1978.) Pp.vii + 285. ISBN-0-08-023761-4. (Oxford and New York: Pergamon Press, 1979.) £22.

HAFNER, H. (ed.). Estimating Needs for Mental Health Care: a Contribution to Epidemiology. Pp.viii + 136. ISBN-3-540-09425-3. (Berlin and New York: Springer-Verlag, 1979.) DM34; $18.70.

HALL, J.L. (ed.). Electron Microscopy and Cytochemistry of Plant Cells. Pp.xii + 444. ISBN-0-444-80135-9. (Netherlands: Elsevier/North Holland Biomedical Press, 1978). US$46.25/Dfl.95.00.

HALTENORTH, Theodor. British and European Mammals, Amphibians and Reptiles. Translated and edited by Gwynne Vevers. (Chatto Nature Guides.) Pp.143. ISBN-0-7011-2364-8. (London: Chatto and Windus, 1979.) Hardback £4.95; Paperback £2.50.

HARLEY J.L. and SCOTT RUSSELL, R. (ed.). The soil-Root Interface. Pp.xx + 448 ISBN-0-12-325550-3. (London: Academic Press Inc. (London) Ltd, 1979). £15.40.

HATHAWAY, D.E. (senior reporter). Foreign Compound Metabolism in Mammals, Vol.5 (A Review of the Literature Published during 1976 and 1977. A Specialist Periodical Report.) Pp.xv + 567. ISBN-0-85186-048-6. (London: The Chemical Society, 1979. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £32; $70.

HAUPT, W. and FEINLEIB, M.E. (ed.). Physiology of Movements. Pp.xviii + 731. ISBN 3-540-08776-1 and ISBN-0-387-08776-1. (Berlin: Springer-Verlag, 1979). DM198 $108.90.

HERWIG, Nelson. Handbook of Drugs and Chemicals Used in the Treatment of Fish Diseases: a Manual of Fish Pharmacology and Materia Medica. Pp.xv + 272. ISBN-0-398-03852-X. (Springfield, Ill.: Charles C. Thomas, Publisher, 1979.) $17.50.

HOMBURGER, F. (ed.). The Syrian Hamster in Toxicology and Carcinogenesis Research. (Proceedings of the Symposium held in Boston, Mass., 30 November-2 December 1977.) (Progress in Experimental Tumor Research, Vol. 24.) Pp.x + 442. ISBN-3-8055-2890- (Basel, London and New York: S. Karger, 1979.) SFr.163, DM195; $97.75.

HOYLE, Fred, and WICKRAMASINGHE, Chandra. Diseases from Space. Pp.196. ISBN-0-460-04357-9. (London, Toromnto and Melbourne: J.M. Dent and Sons, Ltd., 1979.) £6.95 net.

KLEIN, E. Roseland, and KLEIN, Peter D. (ed.). Stable Isotopes. (Proceedings of the Third International Conference.) Pp.xxix + 627. ISBN-0-12-413650-8. (New York and London: Academic Press, 1979.) $39.50.

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ST BARTHOLOMEW'S HOSPITAL
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Applicants with training and/or experience in serological techniques, microbiology and immunology will be considered. The work will involve animal studies, the preparation and purification of microbial antigens, preparation and purification of antisera and immunofluorescent techniques.

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Le Centre de recherches écologiques de Montréal (CREM), un organisme de recherche de l'Université de Montréal, dispose d'un poste d'attaché de recherche en écologie végétale, avec rang d'adjoint, d'agrégé ou de titulaire selon expérience. Le candidat doit posséder un PhD ou un DSc, avoir une bonne connaissance des différents types de végétation de l'est de l'Amérique du Nord, des méthodes d'échantillonnage et d'analyse de la végétation, puis avoir une expérience en photo-interprétation avec une ouverture en pédologie. Ce chercheur, le plus souvent au sein d'équipes pluridisciplinaires, sera responsable de la coordination des recherches et des études en écologie végétale dans le cadre d'inventaires axés sur la caractérisation d'écosystèmes, d'études d'impacts, de projets d'aménagement et de zonage écologiques, etc. La langue de travail sera le français. Salaire selon la convention collective des professeurs. Le curriculum vitae et deux lettres de recommandation doivent être adressés le plus tôt possible par courrier recommandé au Dr Raymond McNeil, CREM, Université de Montréal, CP 6128, Succ. "A", Montréal, Québec H3C 3J7, Canada. Le poste doit être comblé le plus tôt possible à partir du 1er Janvier 1980. W585(A)

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Pharmacologist

Biosciences Research Centre, Epsom

A Pharmacologist is required to investigate novel animal systems that are designed to detect compounds that might be useful for the treatment of allergies. Applicants should have a good first degree and, preferably, relevant experience to PhD level, since they would be expected to contribute ideas and techniques to the work.

The Company offers a competitive salary, a non-contributory pension scheme and free life assurance. Flexible hours are worked on this Site.

For an application form please write to or telephone the **Site Personnel Manager, Beecham Pharmaceuticals Research Division, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ. Telephone: Burgh Heath 53344 (ext 2207). Closing date: December 21, 1979.** 3874(A)

Beecham Pharmaceuticals

THE UNIVERSITY OF TEXAS AT AUSTIN
ASTRONOMY DEPARTMENT
invites applications for
TWO ASSISTANT PROFESSOR POSITIONS

These positions are regular, tenure-track assistant professorships with duties that include both teaching and research. Available research facilities include: the optical telescopes (2.7, 2.1, 0.9, and 0.8 m) of McDonald Observatory, the 2 mile 82 cm interferometer of the University of Texas Radio Astronomy Observatory, the 4.9-m antenna of the Millimeter Wave Observatory, as well as the CDC Cyber 170/750 campus computers.

Applications should include: a description of educational background, teaching, and research experience; a list of all publications; preprints of papers submitted but not yet published; and a statement of current research interests and future plans. Applicants should request at least three scientists who know the applicant personally to send letters of recommendation directly to: P. Vanden Bout, Chairman, Astronomy Department, RLM 15.220, University of Texas at Austin, Austin, TX 78712. Applications and letters of recommendation should be received by 1980 January 15.

The University of Texas at Austin is an Equal Opportunity Employer with an Affirmative Action Program. We encourage and welcome applications from all qualified persons regardless of sex, race, creed, national origin, or non-disqualifying handicap. W583(A)

QUEEN MARY COLLEGE
(University of London)
QMC INDUSTRIAL RESEARCH LTD
MICROBIAL ENHANCEMENT OF OIL RECOVERY
THREE RESEARCH ASSISTANTS

A programme of research is being instituted in collaboration with the Department of Plant Biology on Microbiology to develop *in situ* bacterial methods for the enhancement of crude oil recovery, with particular reference to problems of North Sea reservoirs. In the first phase, sponsored by the Department of Energy, three research assistants are being sought for a three-year period, one at postgraduate and two at post-doctoral levels. It is intended to start the programme as soon as staff can be recruited.

Applicants should have a sound background in microbiology with a knowledge of and an interest in biochemistry and genetics. Experience of working with anaerobic bacteria is desirable but not essential.

The initial salaries (under review) are in the ranges £4,333 to £5,488 plus £740 London Allowance, at postdoctoral level and £3,775 to £4,333 plus £740 London Allowance at postgraduate level.

Applications and requests for further details should be sent to Dr J Hume, QMC Industrial Research Ltd, 229 Mile End Road, London E1 4AA. 3897(A)

THE UNIVERSITY OF TEXAS
HEALTH SCIENCE CENTER AT DALLAS
ANATOMIST

The Anatomy Program in the Department of Cell Biology invites applications for a tenure-track position at the Assistant Professor level. PhD, MD or equivalent, postdoctoral training, and experience teaching human gross anatomy are required.

Responsibilities include teaching in the Human Gross Anatomy Course for first-year medical students and participation in the Department's Graduate Program. Candidates will be expected to conduct independent research in some aspect of biological structure, functional/evolutionary morphology, developmental biology or reproductive biology.

Curriculum vitae, brief description of research program, and names of three referees should be sent to: Dr A P Santa Luca, Faculty Search Committee, Department of Cell Biology, The University of Texas Health Science Center at Dallas, 0.S.C. Texas 75235. The University of Texas Health Science Center at Dallas is an Equal Opportunity/Affirmative Action Employer. M/F/H. W598(A)

UNIVERSITY OF LONDON
INSTITUTE OF NEUROLOGY
POST-DOCTORAL ASSOCIATE

Applications are invited for a position in a research group supported by a 5-year MRC programme grant studying the role of 5-hydroxytryptamine in the brain. The post is suitable for a post-doctoral worker from the neurochemical/pharmacological area. Behavioural or neutritional interests would be an advantage. Salary probably between £5,073 to £6,517 (inc. London weighting). A higher salary may be possible for a particularly well-qualified candidate.

Curriculum vitae with names of 2 referees by 7 January 1980 to Prof G Curzon, Institute of Neurology, 33 John's Mews, London WC1. 3886(A)

UNIVERSITY OF SASKATCHEWAN
DEPARTMENT OF BIOLOGY
POSTDOCTORAL POSITION

Available to investigate the use of liposomes for the uptake of macromolecules and organelles into plant protoplasts. Candidate should have strong background in biochemistry and some knowledge of electron microscopy.

The position is available immediately for two year term (possibly renewable for a third year) at a salary of $C 12,500.00 to $C 13,500.00.

Interested candidates should send curriculum vitae and 3 letters of recommendation to Dr L C Fowke, Dept. of Biology, University of Saskatchewan, Saskatoon, Sask., Canada S7N 0WO. W595(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL
(University of London)
SCHOOL OF PATHOLOGY
POST DOCTORAL RESEARCH ASSISTANT
Research in Chemical Carcinogenesis

Applications are invited for the above post funded by the Cancer Research Campaign for 2 years from 1 January 1980 or as soon as possible thereafter. The work is concerned with various aspects of the malignant transformation of cells by nitrosamides and involves cell culture techniques. A background in Microbiology and Molecular Biology would be advantageous. Starting salary depending on age and experience within the range £4,073 to £6,228 including London allowance.

Applications including a curriculum vitae and the names and addresses of two referees should be sent to Dr J P Roscoe, School of Pathology, The Middlesex Hospital Medical School, Riding House Street, London W1P 7LD. 3879(A)

UNIVERSITY OF REGENSBURG
Germany, Bavaria
POSTDOCTORAL FELLOWSHIP
In Biochemistry

Candidates (PhD in chemistry or biochemistry) should be interested in work on the biosynthesis, structure and function of membrane glycoproteins of halobacteria and eucaryotic microorganisms.

Experience in biochemical methods is essential. Salary will be about DM40,000 p.a. A curriculum vitae, copies of former work and the names of referees should be sent to: Institut für Biochemie, Genetik und Mikrobiologie, Lehrstuhl Biochemie I, Universität Regensburg, Universitätsstraße 31, 8400 Regensburg FRG, Prof. Dr M Sumper. W584(E)

UNIVERSITY OF ABERDEEN
PHYSICIST
(Lecturer)

in Department of Bio-Medical Physics and Bio-Engineering to join radio-nuclide clinical service team and contribute to internationally known programme in radio-nuclide emission tomography. Ability to provide support also for EMI brain and body scanners an advantage. Some teaching duties to MSc (Medical Physics).

Salary on Lecturer Scale £4,333 to £8,992 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 4 January 1980. 3866(A)

THE UNIVERSITY OF NEW SOUTH WALES
Sydney, Australia
SCHOOL OF APPLIED GEOLOGY
LECTURER (GEOSTATISTICIAN)

Duties include the instruction of 3rd and 4th year undergraduates and postgraduate students in the applications of statistics and computers to geology. The successful applicant will be expected to assist in the development of major research programmes in Mineral and Energy Resources (especially in interpretation of exploration data), and in Hydrogeology and Engineering Geology.

Applicants should have a higher degree in a relevant geoscience subject. Teaching experience is an advantage but is not essential; industrial experience is desirable. This position is available on a fixed term contract for three years.

Salary per annum: $A16,291 range $A21,401.

Commencing salary according to qualifications and experience.

Applications close 31 December, 1979.

Full information about conditions of employment and method of application can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3865(A)

CSIRO AUSTRALIA
Research Scientist
Division of Horticultural Research Adelaide

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

GENERAL: The Division, which has laboratories at Adelaide, SA and Merbein, Victoria, conducts applied research on grapevines and a range of subtropical and tropical fruit crops. This is associated with a basic research program on the physiology of horticultural crops. Current programmes of the Division that are relevant to this position include research on the biogenesis of higher plant chloroplasts, anatomical investigations of plant parasitic nematodes, ultrastructural studies of the pollination process of selected perennial plants, and EDAX studies of the distribution of inorganic elements in the organelles of plant cells. Facilities: The Division has a high resolution electron microscope capable of being used in both the scanning and transmission modes. It is equipped with EDAX facilities. Also available is freeze-etch equipment and a scanning electron microscope.

LOCATION: The appointee will be located at the Adelaide Laboratory.

DUTIES: An experienced cell biologist with specialist skills in light and electron microscopy is sought to work in the broad area of the developmental anatomy of higher plants. The appointee would be expected to bring to the Division expertise in ultrastructural and associated techniques and to develop an independent research programme.

QUALIFICATIONS: A PhD degree or equivalent with experience of light microscopy and of transmission and scanning electron microscopy. Familiarity with EDAX and/or freeze-fracture techniques would be an advantage.

TENURE: Indefinite with superannuation.

SALARY: Senior Research Scientist/Principal Research Scientist: A$20,198 — A$27,212 pa.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 491/058 should reach:-

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 5th January 1980.

Applications in USA and Canada should be sent to:- The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, NW, Washington, DC, 20036, USA 3895(A)

Applications are invited for a position at the EUROPEAN MOLECULAR BIOLOGY LABORATORY in Heidelberg, Federal Republic of Germany, as
Technical Assistant

The successful applicant will work on the isolation and analysis of nucleic acid from bacterial and animal viruses, but some experience of microbiology and/or cell biology will be useful. Familiarity with chromatographic and electrophoretic methods and use of radioactive isotopes is essential.

Applicants should have had appropriate technical training together with experience in the above techniques. Knowledge of English and German is essential.

The salary offered to the successful candidate will be between DM2200 and DM2600 monthly, in addition to which certain allowances are payable, depending on personal circumstances.

Please write briefly for an application form, quoting reference no: 79/22, to:

EMBL, Personnel Section, Postfach 10.2209, D — 6900 Heidelberg, Germany. W589(A)

Opportunities in Metabolic Studies

G.D. Searle is a leading international Pharmaceutical Company with its main U.K. headquarters at High Wycombe in Buckinghamshire. We currently have vacancies for a Mass Spectroscopist and a Biochemist in our Metabolic Studies Department, which is involved in studies of the metabolism and pharmacokinetics of drugs in animals and man.

Mass Spectroscopist

(Ref. MS/R52)

A Mass Spectroscopist is required to supervise the Mass Spectrometry group, which provides g.l.c. and g.c.-m.s. support for metabolism studies and provides a service to synthetic organic chemists for structure elucidation of compounds undergoing research and development. This post would suit a PH.D Chemist with experience in the application of g.c.-m.s. to the analysis of drugs and metabolites in biological fluids.

Biochemist

(Ref. B/R54)

A Biochemist is required to assist in carrying out drug metabolism studies in experimental animals and man. Applicants should have a degree or equivalent qualifications in Biochemistry or Biochemical Pharmacology, and should preferably have some experience of radioisotope and chromatographic techniques. Some experience of animal work would also be of value.

We offer competitive salaries, and our terms of employment include 4 weeks' holiday per year, contributory pension scheme, private health insurance scheme, sports and social club, and subsidised cafeteria.

Please apply, quoting the appropriate reference, to Miss D. Wardman, Assistant Personnel Officer, Searle Research & Development, Lane End Road, High Wycombe, Bucks (Tel. High Wycombe 21124 ext. 3254). 3884(A)

SEARLE

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

RESEARCH FELLOW — DEPARTMENT OF BIOCHEMISTRY

The successful applicant will hold a PhD or have equivalent experience, and will be required to undertake research on the mechanism of enzyme reaction within a group headed by Dr J F Morrison. Current projects are concerned with the chemical mechanism of the reaction catalyzed by chorismate mutase-prephenate dehydrogenase, the slow-tight-binding inhibition of dihydrofolate reductase by folate analogues and the inhibition of phosphotransferases by lanthanide-nucleotide complexes. Applicants should have had postdoctoral experience in enzymology and possess expertise in kinetic and other procedures for studying enzymic catalysis.

The appointee should be able to commence duty in the first half of 1980.

CLOSING DATE: *31 JANUARY 1980.*

RESEARCH FELLOW — DEPARTMENT OF PHYSICAL BIOCHEMISTRY

Applicants should hold a PhD or have equivalent experience and be interested in joining a group engaged in one of the following fields of research: the physical and chemical characterization of biological macromolecules, interacting protein systems including studies on subunit assembly, self-associations and acceptor-ligand binding (Professor L W Nichol and Dr P J Jeffrey); studies of protein hydration and denaturation primarily involving calorimetric and spectroscopic methods (Dr H A McKenzie); comparative studies of sequences and properties of transferrins, lactoferrins, a-lactalbumins and lysozymes (Dr H A McKenzie and Dr D C Shaw). Further information may be obtained from Professor L W Nichol (Head of Department), in the University.

The appointee should be able to commence duty in the first half of 1980.

CLOSING DATE: *31 JANUARY 1980.*

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES POSTDOCTORAL FELLOW — DEPARTMENTS OF DEVELOPMENTAL BIOLOGY AND GENETICS

The Departments are undertaking a joint program on the role of phytohormones in the *Rhizobium*-legume symbiosis. The successful applicant will work with Dr D S Letham and associates on the determination of the phytohormones produced by different *Rhizobium* mutants and with Drs J Shine and B Rolfe on the cloning of genes responsible for their synthesis. Preference will be given to applicants with experience in biochemistry and/or molecular biology. Skill in the handling of bacteria would be an advantage.

CLOSING DATE: *30 JANUARY 1980.*

POSTDOCTORAL FELLOW — DEPARTMENT OF NEUROBIOLOGY

The successful applicant will be familiar with immunocyto-chemical techniques for the ultrastructure localisation of cytoskeletal proteins, and will pursue research on the architecture of the photoreceptor microvilli of arthropods in collaboration with Dr A D Blest. Interaction with studies of plant cyto-architecture directed by Professor B E S Gunning is also anticipated.

CLOSING DATE: *15 JANUARY 1980.*

SALARIES: Salary on appointment will be in accordance with qualifications and experience as follows: Research Fellow $A16,291-$21,265; Postdoctoral Fellow: a fixed point between $A16,291-$21,265 per annum. Present exchange rate: $A1: UK.52p.

TERM OF APPOINTMENT: Research Fellow, John Curtin School of Medical Research — two years, but in exceptional circumstances, three years with possible extension to five years. Postdoctoral Fellow — one to two years, although persons from overseas are not normally appointed for less than two years.

OTHER CONDITIONS: Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should first obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 3890(A)

UNIVERSITY OF OXFORD
DEPARTMENT OF ZOOLOGY

DEPARTMENTAL DEMONSTRATORSHIPS IN ZOOLOGY

Applications are invited for two Posts of Departmental Demonstrator in Zoology, with special reference to Vertebrate Zoology and Animal Behaviour. The persons appointed would be expected to take up their posts by October 1980. The successful candidates, who should have recently completed a Degree of Doctor of Philosophy or be in the final year before submission, will be required to take part in Zoology teaching and to undertake research in Zoology.

The appointments will be for three years in the first instance, with the possibility of renewal for a maximum of a further three years. The salary (subject to review) will be within the University Scale £3,775 to £5,199 per annum, with membership of USS.

Applications, with the names of two referees, should be submitted to the Head of Department, Department of Zoology, South Park Road, Oxford, OX1 3PS, by 10th January 1980. 3893(A)

POLYTECHNIC OF THE SOUTH BANK

DEPARTMENT OF ELECTRICAL ELECTRONIC ENGINEERING

RESEARCH ASSISTANT

Required to work on the development of the new design methods for Analogue/Digital Electronic Filters.

Salary £3,321 to £3,705 (inclusive of Inner London Allowance). Enquiries to Dr J K Stevenson (01-928 8989 Ext 2273).

Further particulars and application forms from the Staffing Officer, Polytechnic of the South Bank, Borough Road, London SE1 0AA. 3860(A)

POSTDOCTORAL POSITIO starting January 1980 to study molecul biology of mammalian mitochondri DNA. Experience in recombinant DN cloning and DNA sequencing is desirab but not necessary. Send curriculum vitae, brief summary of past research and nam of references to either Dr. P. J. Laipis Dr. W. W. Hauswirth, Department Biochemistry and Molecular Biology, Bo J-245, JHMHC, University of Florid Gainesville, Florida 32610. *Equ Employment Opportunity/Affirmati Action Employer* W605(

UNIVERSITY OF DUBLIN Trinity College

UNIVERSITY CHAIR OF BOTANY (1711)

This Chair will fall vacant on 1st October 1980, when Professor W A Watts, at his own request, transfers to a Personal Chair.
Further particulars of the post may be obtained from:

Mr G H H Giltrap
Secretary to the College
West Theatre
Trinity College
Dublin 2
Tel. 772941, Ext. 1123

to whom completed applications should be sent preferably by *Friday, 25th January 1980.* 3888(A)

UNIVERSITY OF REGINA

DEPARTMENT OF BIOLOGY

Applications are invited for the post of

RESEARCH STUDENT (MA or PhD)

the area of genetics of grasshoppers ntrol and populational aspects). Support funds available. Applications, luding curriculum vitae and names of references should be addresed to W Chapco, Dept. of Biology, iversity of Regina, Regina, katchewan, Canada S4S 0A2. W602(A)

JERRY LEWIS MUSCLE RESEARCH CENTRE

POST-DOCTORAL RESEARCH FELLOW

ired to work in collaboration with David Shotton, Department of hemistry, Imperial College, London, freeze fracture investigations of rophic human muscle. Previous rience in muscle research and electron oscopy desirable. Post tenable for 3 s, funded by the Wellcome Trust. ting salary £5,488 plus £740 London wance per annum.
pplications, with a curriculum vitae, a statement of research interests and rience, and names and addresses of 2 rees, to Professor V Dubowitz, rtment of Paediatrics and Neonatal icine, Institute of Child Health, mersmith Hospital, Du Cane Road, lon, W120HS. 3892(A)

UNIVERSITY OF CAPE TOWN

LECTURESHIP IN ORGANIC CHEMISTRY

ications are invited for the above post, nt as from 1st July 1980. ointment, according to qualifications xperience, will be made on the salary R8 100 × 450 — 9000 × 600 — R13200 nnum.
plicants should submit a curriculum stating present salary, research sts and publications, the date duty be assumed, and the names and sses of three referees.
moranda concerning the position and l conditions of service should be ed from the Registrar, Room 10, rsity of Cape Town, Private Bag, bosch 7700, by whom applications e received not later than 31 March W592(A)

WASHINGTON UNIVERSITY

School of Medicine

DEPARTMENT OF MEDICINE

Applications are invited for a

POSTDOCTORAL ASSOCIATE

to work on the biosynthesis of pancreatic islet polypeptide hormones. Experience in protein or nucleic acid chemistry is highly desirable. Salary approximately $15,000.

Applications, including a full c.v. and the names of two academic referees should be sent to Dr. Alan Permutt, Department of Medicine, Washington University School of Medicine, 660 S. Euclid, St. Louis, Missouri 63110, USA. W593(A)

McGILL UNIVERSITY

POSTDOCTORAL POSITION: CELL BIOLOGY

Applications are invited for a postdoctoral position for 2 years. The post is for research in the regulation of DNA replication and functions of tumor antigens in mammalian cells. It is available April 1, 1980. Previous experience in cell culture is required and training in molecular biology, animal virology or somatic cell genetics would be helpful. The research group includes other postdoctoral fellows and graduate students. Opportunities are available for interaction with other groups interested in nucleic acid research.

Applications with curriculum vitae and names of reference to: Dr Roger Hand, McGill Cancer Centre, 3655 Drummond, Montreal, Que. H3G 1Y6. W575(A)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

LECTURER: MOLECULAR GENETICS

Applications are invited for the post of Lecturer, in Molecular Genetics, in The School of Biological Sciences. Applicants should have research interests in molecular genetics and experience in modern methods of gene manipulation and DNA biochemistry. The salary will be within the scale £4,333 to £8,992 (under review) with superannuation benefits.

Application forms and further particulars can be obtained from the Assistant Secretary of Science, Science Office (E), Sussex House, University of Sussex, Falmer, Brighton BN1 9RH, to whom applications (8 copies) should be sent by 14th January 1980. 3822(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Forest Research Kelmscott Western Australia

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of who are research and professional scientists — located in Divisions and Sections throughout Australia.

FIELD: Plant Pathology

DUTIES: The appointee will be part of a team of CSIRO and Western Australia Forest Department scientists who are studying eucalypt dieback in the native jarrah forests. Research will be primarily directed toward an understanding of those factors of the soil environment which affect disease incidence.

QUALIFICATIONS: Applicants should possess a PhD or equivalent and have research experience in soil-borne plant diseases or soil microbial ecology.

TENURE: Up to three years with a possibility of further extension. Superannuation benefits available.

SALARY: Research Scientist/Senior Research Scientist: A$15,916 — A$23,122 pa.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 1050/138 should reach:-

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 5th January 1980.

Applications in USA and Canada should be sent to:- The Counsellor Scientific, Embassy of Australia, 1610 Massachusets Avenue, N.W., Washington, D.C., 20036, USA. 3896(A)

THE CHINESE UNIVERSITY OF HONG KONG

FACULTY of MEDICINE

Applications are invited for the following post:

DIRECTOR OF THE ANIMAL HOUSE

tenable at this new medical school. The Animal House of the Faculty of Medicine will ultimately become the Main University Animal House.

Qualifications

Applicants should be qualified veterinary surgeons, preferably with relevant experience in the management of animal house in academic/research institutions.

Date of Assumption of Duty

The appointee is expected to assume duty by 1st January 1981 to supervise installation of facilities. Teaching in this Faculty will commence in the fall term of 1981.

Conditions of Service

Salary: HK$105,540 — 141,780 per annum by 8 increments. (Exchange rate approximately £1 = HK$10.8) Terms of service include long leave, superannuation benefits (University 15%, appointee 5%), medical and housing benefits and for overseas appointees, passage benefit as well.

Application Procedure

Application forms and further information on the Faculty and the scope of the job are obtainable from the Personnel Section, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. Closing date of application is *31st March, 1980.* W603(A)

KUWAIT

UNIVERSITY OF KUWAIT FACULTY OF MEDICINE
MEDICAL SCIENCE AND CLINICAL APPOINTMENTS

Applications are invited for the following appointments in the Faculty of Medicine:-

ANATOMY

Associate professor. Candidates should have areas of specialisation in electron microscopy. The successful candidate's responsibilities will include directorship of the E.M. Unit which is equipped with scanning and transmission microscopes.

Assistant or associate professor. Preference will be given to candidates who have experience in teaching the anatomical disciplines to allied health students. The successful candidate will be responsible for coordinating allied health teaching in the Department of Anatomy.

COMMUNITY MEDICINE

Assistant or associate professor in epidemiology (2 posts).

Assistant or associate professor in public health.

Preference for these posts will be given to suitable candidates who speak Arabic.

MEDICINE

Associate professor in nephrology.

Associate professor in neurology.

OBSTETRICS AND GYNAECOLOGY

Associate professor or professor, and assistant professor (2 posts).

Assistant professor or associate professor. Scientist gynaecologist conversant with the practical aspects of gynaecologic endocrinology and hormone assay. A biochemist with extensive experience in these areas may apply and, if appointed, would also be expected to undertake some teaching in the Department of Biochemistry.

PATHOLOGY

Assistant and associate professors (2 posts). Candidates should have experience in academic pathology, particularly in anatomic and histopathology. The successful candidates will have responsibilities in diagnostic pathology at the teaching hospital.

PHARMACOLOGY

Professor. Candidates should be medically qualified.

PHYSIOLOGY

Associate professor or professor in neurophysiology.

Assistant or associate professor in general physiology. Preference will be given to candidates with specialisation in one of the following areas: cardiovascular, gastro-intestinal, muscle physiology, haematology.

PSYCHIATRY

Associate professor or professor. Preference will be given to suitable candidates who speak Arabic.

SURGERY

Associate professor or professor in orthopaedic surgery.

Associate professor in E.N.T. surgery.

Assistant or associate professor in plastic, reconstructive and burn surgery.

Assistant or associate professor in general surgery with special interest in traumatology.

Assistant professor in surgical research.

Applicants for surgical posts should have at least 5 years clinical and teaching experience in their respective specialties.

Conditions of appointment

Applicants should possess a PhD, or higher professional qualification in their respective medical or surgical specialty, and have conducted and published research in their field. Professors should have 14 years' experience, 4 as an associate professor or its equivalent, i.e. senior lecturer or reader. Associate professors should have 9 years' experience, 4 as an assistant professor or its equivalent i.e. lecturer.

Salaries: Total monthly salaries will be within the following scales according to qualifications and experience (1KD = £1.60; US$3.6 approx.).
Professors with clinical appointments: KD 880-1045 (8 increments).
Professors medically or non-medically qualified with medical science appointments: KD 826-982 (8 increments).
Associate professors with clinical appointments:
KD 725-890 (8 increments).
Associate professors medically qualified with medical science appointments: KD 680-836 (8 increments).
Associate professors non-medically qualified: KD 650-800 (8 increments).
Assistant professors with clinical appointments:
KD 570-735 (8 increments).
Assistant professors medically qualified with medical science appointments: KD 534-690 (8 increments).
Assistant professors non-medically qualified: KD 510-660 (8 increments).

Clinical supplements: In addition to the above university salaries there will be a monthly clinical supplement paid by the Ministry of Public Health for medical school staff with clinical service commitments. These are:

Professor and chairman	— KD 250
Professor	— KD 200
Associate professor	— KD 150
Assistant professor	— KD 100

Gratuity: There is a gratuity of one month's basic salary for each year employed payable on termination of contract.

Housing: Suitably furnished, air-conditioned accommodation, electricity and water are provided free of charge.

Medical care: Free, comprehensive treatment is available in Kuwait under the State Health Service.

Travel: Air tickets are provided from the country of recruitment for the appointee, spouse and up to 3 dependent children under 20 years. Thereafter return air tickets are issued annually to the country of citizenship or permanent residence. On termination of contract, tickets are provided to the country of recruitment. A baggage and freight allowance is also provided. In addition, travel expenses for attending one scientific meeting a year are paid by the University.

Vacation: Sixty days paid annual leave and various official holidays.

Education: this is provided free in state schools where the instruction is in Arabic. Staff who have to send their children to non-Arabic schools in Kuwait will have the tuition fees of up to a maximum of three met by the University.

Taxation: There is no income tax in Kuwait. Currency is freely transferable without restriction.

Method of application

Curriculum vitae in duplicate, which should include the names of three referees, personal particulars, qualifications with dates, career history, teaching experience, research accomplishments and, where appropriate, clinical experience should be sent to the Dean, Faculty of Medicine, University of Kuwait, P.O. Box 24923 Safat, Kuwait to arrive no later than 31st January 1980. W604(A)

CANCER RESEARCH UNIT, University of Western Ontario, London, Ontario. Positions are available for staff at the Assistant or Associate Professor level (non-tenure track) and for postdoctoral fellows and graduate students. Research is focussed on the areas of cellular and viral DNA replication, nucleic acid metabolism in eukaryotic cells, mRNA cloning, and mechanisms of cell transformation.

The unit is well funded by the National Cancer Institute of Canada and is affiliated with the Department of Biochemistry and the Department of Microbiology and Immunology in the Medical School of the University.

Candidates for a staff position should submit a curriculum vitae, statement of research interests, and the names of three referees to Dr. David T. Denhardt, c/o The Administrative Secretary, Cancer Research Unit, University of Western Ontario, London, Ontario N6A 5B7, Canada. Students should apply for admission through the above mentioned Departments. W[illegible]

MEDICAL LABORATORY SCIENTIFIC Officer, associate IMLS or equivalent, experience in radioimmunoassay techniques an advantage. Appointment is for three years. Salary in the range £4122 to £4620 depending on experience. Applications giving the names of two referees to Professor L P Le Quesne, Department of Surgical Studies, The Middlesex Hospital Medical School, Mortimer Street, London W1N 8AA. 3877(A)

BIOZYME LABORATORIES LTD
Unit 6, Gilchrist-Thomas Estate,
Blaenavon,
Gwent NP4 9RL

MANUFACTURERS OF ENZYMES AND RELATED BIOCHEMICALS

require

(1) PRODUCTION CHEMISTS

and

(2) PRODUCTION CHEMICAL TECHNICIANS

To be employed to carry out varied and interesting work in the Production and Analytical Department.

Experience, though helpful, is not essential, but minimum qualifications should be
(1) BSc or equivalent with Chemistry as major subject
(2) 'A' Level or equivalent in Chemistry.

Training will be given to suitable applicants in the various departments and the positions carry excellent promotional prospects.

Please apply for application form. 3894(A)

ELECTROPHYSIOLOGIST BIOPHYSICIST research associate t study membrane currents under voltag clamp in dialyzed and intact neurones Emphasis on mechanisms of chemica modulation of conductances. Applicant should have relevant training plus goo physical background, preferably wit digital computer experience. Appointmen for two years or more to begin as soon a possible. Send CV and names of tw referees to Dr R Eckert, Department c Biology, University of California, L Angeles, CA 90024. Equal Opportunity Affirmative Action Employer. W596(A)

INSTITUTE OF CANCER RESEARCH

ULTRASONIC IMAGE ANALYSIS IN CANCER INVESTIGATION

A PHYSICAL SCIENTIST

is required to help co-ordinate the work an active, multi-disciplinary team applying pattern recognition and ima analysis techniques to the extraction diagnostic information from ultrason B-scan images. The work forms part of larger research programme in ultrasour jointly organised between Physics ar Clinical Diagnostic groups at the Institu of Cancer Research and Royal Marsde Hospital, Sutton, Surrey, and the prese project involves collaboration with thr other major laboratories in the fields ultrasonic imaging and data analysis.

Candidates should preferably ha experience in ultrasound or image analys methods and should hold a PhD in appropriate field, or be able demonstrate a comparable level of attai ment. The appointment will be for thr years in the first instance.

Salary will be on MRC Scientist scal (grade II, non-clinical) commencing at point in the range £4,333 p.a. to £5,777 p. depending on qualifications ar experience. In addition London Allowan of £740 p.a. is payable, and the post carri superannuation under the USS pensi scheme.

Applications with full curriculum vi in duplicate and the names and addresses two professional referees should be sent the Secretary, Institute of Canc Research, 34 Sumner Place, London, S 3NU, quoting ref. 300/B/20 3901(A)

Southampton THE UNIVERSITY

OCEANOGRAPHY DEPARTMENT

A RESEARCH ASSISTANT

is required by the Department of Oceanography, Southampton University, for a one year project to investigate applications of LANDSAT imagery in oceanography. Applicants must have a degree in physical/chemical oceanography and be experienced in the interpretation and enhancement of LANDSAT imagery.

Salary on scale 1B from £3775 per annum.

Applications giving date of birth, brief curriculum vitae and the names of two referees to Mrs E C P Sears, Staffing Department, The University, Highfield, Southampton SO9 5NH, within one week of the appearance of this advertisement. Please quote reference number 1904/R/N. 3868(A)

BRIGHTON POLYTECHNIC
DEPARTMENT OF PHARMACY
POST-DOCTORAL RESEARCH FELLOW

A post funded by the Medical Research Council is available for a research fellow to carry out a biophysical study of cervical mucus thickening. The post would suit persons holding higher degrees in pharmacy, biochemistry, biophysics or any other related subject.

Appointments will be made on an annual basis up to a maximum of three years and the initial salary will be £4,083 (+£72 per annum pending comparability study).

Applications, in the form of a letter, together with a curriculum vitae and the names of two referees, should be made by 31 January 1980 to the supervisor, Dr C Marriott, Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ, Tel Brighton 693655 Ext 2213. 3891(A)

MEDICAL RESEARCH COUNCIL
LABORATORY OF MOLECULAR BIOLOGY

pplications are invited for a scientific pointment, available from September, 80.

The post is a permanent one and plicants must have had extensive earch experience and accomplishment in e field of the immunobiology of haema-oeisis.

Salary scale £8,698 to £10,775.

Applications, with full c.v. and names of o referees, should be sent to The ministrative Secretary, Laboratory of olecular Biology, The Medical School, ls Road, Cambridge CB2 2QH. 3881(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
BIOCHEMIST OR PHARMACOLOGIST

search Assistant required at tgraduate or postdoctoral level to work mechanism of action of nicotine-like gs. Appointment at appropriate ition on University scale.

nowledge of membrane or lipid chemistry an advantage.

lease apply in writing to Dr J M leton, Department of Pharmacology, g's College, London WC2R 2LS. 3817(A)

BIOGEN S.A.

a young company engaged in basic and applied research in the field of molecular biology and microbiology, and with the objective of utilizing the potential of genetic engineering for the production of substances of commercial and medical interest in such areas as health care, agriculture and energy, is inviting applications for its **Research Laboratory in Geneva**, Switzerland:

RESEARCH MANAGER, for a person of

- outstanding scientific level in the field of molecular biology and microbiology,
- with proven ability in the coordination and administration of the activities of a research laboratory,
- sense of human relations, as well as capacity for organization, are required.

NUCLEIC ACID CHEMIST

with good experience in recent technologies for the synthesis of DNA.

In both cases, salary (including fringe benefits) will be competitive and in accordance with competence and experience. Send curriculum vitae (with a description of previous experience and a list of publications) to BIOGEN Laboratory, 3 Route de Troinex, 1227 Geneva, Switzerland. W590(A)

UNIVERSITY OF NEWCASTLE UPON TYNE
THE MEDICAL SCHOOL
LECTURESHIP IN ANATOMY

Applications are invited from registered medical practitioners or other suitably qualified candidates for the post of Lecturer in Anatomy to be filled as soon as possible. The Lecturer will be expected to devote the whole of his/her time to teaching and research work in the Department of Anatomy. There are good facilities for research in the Department, including developmental cell biology, immunology, neurophysiology, kinaesiology, neuroanatomy, general histology and electron microscopy.

Some preference would be given to a candidate whose research interest lies within developmental cell biology and/or immunology and who has experience of teaching gross anatomy.

Salary will be at an appropriate point on one of the following scales according to age, qualifications and experience: Lecturers' Non-Medical £4,333 to £8,992 per annum; Lecturers' Pre-Clinical General £5,777 to £8,992 per annum; Lecturers' Pre-Clinical Higher £8,404 to £9,581 per annum. Membership of the appropriate University Superannuation Scheme will be required.

Further particulars may be obtained from the Senior Assistant Registrar (FP), The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU with whom applications (3 copies) together with the names and addresses of 3 referees, should be lodged not later than 2nd January, 1980. Please quote reference N. 3870(A)

BRITISH POSTGRADUATE MEDICAL FEDERATION
(University of London)

CARDIOTHORACIC INSTITUTE
(Associated with National Heart and Chest Hospitals)

NON-CLINICAL SENIOR LECTURER IN CARDIAC MUSCLE RESEARCH UNIT

A vacancy occurs for a non-clinical Senior Lecturer in the Department of Cardiac Medicine at 50 Wimpole Street, London W.1. The Department has an active research unit in myocardial metabolism, with a team of 15 graduates and programmes in: ion transport, mitochondrial, plasmalemmal and sarcoplasmic reticulum function, electrophysiology, adrenergic receptors, ultrastructure, and the pathophysiology of hypoxia, ischaemia and experimental cardiomyopathy.

The successful candidate would be expected to contribute to or widen the activities of the Unit and to take an important share in the direction of the research. Applications are invited from scientists with a well-established interest in the fields of myocardial biology, biochemistry, physiology or pharmacology.

Salary will be on an appropriate point on the non-clinical Senior Lecturer scale, currently £8,698 to £10,775 + £740 London Allowance. The post is superannuable in the USS.

For further information please contact the Head of the Department, Professor Peter Harris, telephone 01-486 3043, who will be glad to discuss the nature of the appointment.

Formal applications, in duplicate, including curriculum vitae, list of publications and names of three referees, should reach the Secretary, Cardiothoracic Institute, Fulham Road, Brompton, London SW3 6HP not later than 11th January 1980. 3900(A)

European Molecular Biology Laboratory

INSTRUMENTATION DIVISION

The Instrumentation Division of the EMBL is responsible for the development and implementation of advanced instrumentation and experimental techniques in the fundamental biological sciences. Applications are invited from suitably qualified and experienced applicants to fill the following vacancies at the new EMBL Laboratory in Heidelberg, Federal Republic of Germany.

NUMERICAL DATA ANALYSIS
Mathematician/Programmer (Ref. 79/14)

to develop new algorithms in such areas as image processing, large-scale constraint optimisation and statistical data analysis, X-ray diffraction; also to implement the algorithms in portable user-oriented programs.

CHEMICAL INSTRUMENTATION
Research Assistant (Ref.: 79/17)

to design, develop and adapt laboratory instrumentation for new instrumental techniques (eg stopped-flow kinetics, sequence analysis etc.). This work will be carried out in close collaboration with one or more of the biological groups which are active in these areas.

ELECTRON MICROSCOPY
Physicist (Ref.: 79/24)
Technical Assistant (Ref. 79/25)

The EM Development Group is responsible for the design and construction of two new electron microscopes as well as the development of techniques and ancilliary equipment for the existing microscopes at EMBL. The successful applicants for the above posts will take part in one or more aspects of these activities.

The initial contract offered to successful applicants will be of three years' duration. The salary offered will be between DM3000 and DM4000 for the mathematician/programmer, research assistant and physicist and between DM2600 and DM3300 for the technical assistant post. The monthly salaries quoted are net of tax and will be augmented by allowances for expatriation and family, depending on individual circumstances.

Please apply briefly to:

EMBL, Personnel Section, Postfach 10.2209, D — 6900 Heidelberg, Federal Republic of Germany, for an application form, **quoting the reference number** of the post which interests you. W588(A)

SCIENTIFIC & MEDICAL PUBLISHING

EDITORIAL SERVICES & PRODUCTION CONTROLLER

Macmillan Scientific and Medical Division, based in Basingstoke, publishes both books and journals in high-level sciences and medicine.

A new position has been created within the division for a person to handle the book publishing programme from receipt of manuscripts to production of printed copies.

The work involves close liaison with top academic authors, organisation of the editing process and regular contact with printers. Applicants should be familiar with copy-editing, proof reading and the preparation of artwork and an appreciation of modern printing techniques is desirable. A god command of English, a concern for detail and the ability to work under pressure are essential. A scientific or medical background would be helpful.

Applications are invited from candidates with relevant publishing experience. This is a senior position within the division and commands a competitive salary and attractive benefits.

Please write for full details to Sheilagh Browne, Macmillan Publishers Limited, Bruenl Road, Basingstoke, RG21 2XS. 3898(A)

APPLICATIONS ARE INVITED FOR the position of chairman of the computer science department at Concordia University. The department has over 20 faculty members and a number of system analysts. It offers programmes at both the undergraduate and graduate levels. At the undergraduate level it offers a Batchelor of Computer Science Programme with options in General Science, Digital Systems, General Businees and Mathematics and an Honours programme in Computer Science. There are over 625 undergraduate students, and 120 graduate students, full-time and part-time. The language of instruction is English. Interested candidates may send a curriculum vitae along with the names of three referees to: Prof M N S Swamy, Dean of Engineering, Concordia University, 1455 de Maisonneuve Blvd. West, Montreal, Quebec, Canada, H3G 1M8 W599(A)

THE POLYTECHNIC OF CENTRAL LONDON

SCHOOL OF ENGINEERING AND SCIENCE

MICROBIOLOGY TECHNICIAN GRADE 5

Salary: £4,224 to £4,845 increasing to £4,452 to £5,109 at 1 April 1980. These figures include £525 London Allowance.

Work involves organisation and daily running of Microbiology Teaching Laboratories, preparation of Media, upkeep of stock cultures, maintenance and operation of equipment. Applicants should have a sound knowledge of Microbiology.

The following qualifications are required: HNC, HND, C&G Part III with 8-9 years experience (inclusive of training) or the equivalent and/or appropriate industrial experience.

Application form and job description from the Establishment Officer, PCL, 309 Regent Street, London W1R 8AL (Tel: 01-580-2020 ext. 212). 3872(A)

DALHOUSIE UNIVERSITY
Halifax, Nova Scotia
Canada B3H 4J1
DEPARTMENT OF OCEANOGRAPHY

PHYSICAL OCEANOGRAPHERS

Grant-supported positions available for two research associates for theoretical and experimental work on shelf processes. PhD required. Both post-doctoral and senior applicants welcome. Submit applications with names of three referees before Dec. 15. Further details available. Dr Chris Garrett, Dept. of Oceanography, Dalhousie University, Halifax, N.S., Canada, B3H 4J1. W594(A)

DIRECTOR OF BROOKLYN BOTANIC GARDEN

The Board of Trustees invites applications from candidates having these qualifications:

Demonstrated leadership in imaginative planning, decision making and administration of the scientific, educational and community service functions of a major botanical garden. *Professional training* (preferably earned doctorate) in or related to botany or horticulture. *Understanding of fiscal management* of non-profit organizations and ability effectively to represent the institution in seeking private or public support. Send resumé, bibliography and references to:

Robert A Lehman, Chairman, Search Committee Board of Trustees, Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn, NY 11225.

W580(A)

QUEEN MARY COLLEGE (University of London)

RESEARCH LABORATORY TECHNICIAN GRADE 3

required in the Department of Plan Biology and Microbiology to work unde supervision of Professor E A Bevan Experience in Microbiology and/o Virology with emphasis on Nucleic Acic Techniques desirable. (Temporar appointment for 3 years.)

Salary scale £3,122 to £3,553 p.a. plu £780 p.a. London Weighting. (Salary scal under review, and in addition, will increas by 6½% from April 1980.) 7½ week annual leave including Public Holidays.

Letters only to Establishment Office P/T, Queen Mary College, Mile End Road London E1 4NS stating age, experience an qualifications. 3885(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENTS OF PHARMACOLOGICAL SCIENCES AND CLINICAL BIOCHEMISTRY

RESEARCH ASSOCIATE

Applications are invited from candidat with an interest in metabolic biochemist for a Post-Doctoral post of Researc Associate to participate in a project on t toxicology of compounds which influen intermediary metabolism, directed by Dr S A Sherratt and Professor K G M Alberti, which is supported by t Wellcome Trust. The post is available f three years from 1st April, 1980. Starti salary £4,333 per annum on the Range scale with membership of USS.

Applications, naming two referee should be submitted as soon as possible Dr H S A Sherratt, Department Pharmacological Sciences, The Medi School, University of Newcastle up Tyne, Queen Victoria Road, Newcas upon Tyne NE1 7RU from whom furth information can be obtained, tel. 06 28511 ext. 3031. 3862(A)

UNIVERSITY OF OXFORD
in association with University College
READERSHIP IN THEORETICAL PHYSICS

Applications are invited for the above post tenable in the Department of Theoretical Physics to be filled from 1 October 1980. Stipend on the scale £8,698 to £10,775 per annum (under review). The appointment may be held in conjunction with a Fellowship at University College.

Further details may be obtained from Professor R J Elliott, FRS, Department of Theoretical Physics, 1 Keble Road, Oxford, OX1 3NP, to whom a single application for both the University and college appointments, with a curriculum vitae and the names of two referees, should be sent by 31 January 1980. 3861(A)

UNIVERSITY OF EDINBURGH

POSTGRADUATE RESEARCH ASSOCIATESHIP IN STEROID BIOCHEMISTRY

Applications are invited from candidates with a Biochemical-Physiological training for the above position in the Department of Surgery, to investigate the metabolism of androgens in the cellular components of the human prostate. The grant is for a period of 3 years and the starting salary will be £3,883 on the range 1A.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr F K Habib, Department of Surgery, University of Edinburgh, Medical School, Teviot Place, Edinburgh, EH8 9AG. Please quote Reference 5067. 3880(A)

RESEARCH OFFICER/ TECHNICIAN

to assist in our Cell Surface Biochemistry Laboratory on the isolation and structure of lymphocyte surface membrane proteins. HNC/degree in chemistry, biochemistry or related subject. Practical experience will be an advantage.

Salary range £4,523 to £6,500. For further information and application form write or telephone Miss S M Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2 on 242 0200 ext 305, quoting ref. CSB. 3887(A)

HOUGHTON POULTRY
RESEARCH STATION
SCIENTIFIC OFFICER HISTOLOGY

Vacancy exists in the Histology Section a scientific officer to assist with the vision of routine histological services the performance and development of cialised techniques.

Applicants must have a degree, HNC or ivalent qualification with several years' erience in histology.

The appointment will be to the Scientific cer grade (£3,289 × 12 to £5,085) ch will be revised to (£3,591 × 10 to 486) with effect from 1st January 1980 a starting salary depending upon lifications and experience. Non-tributory superannuation scheme.

Further particulars and application ms from the Station Secretary, ghton Poultry Research Station, ghton, Huntingdon, Cambs. PE17 . Closing date for application is 20th ember 1979. 3852(A)

unilever research

Physical Chemist

We have a vacancy for a Physical Chemist in our Ice Cream and Dairy Section. The person appointed will work on background research into the mechanisms involved in the stabilisation and destabilisation of food emulsions. In particular he/she will (a) investigate the interactions between emulsifying and stabilising agents adsorbed at the oil/water interface and their effect on the physical properties of emulsions and (b) study the adsorption and desorption behaviour of emulsion droplets to the air/water interface and thereby develop an understanding of the effect of aeration on emulsion stability.

Candidates should have a degree or equivalent in chemistry and experience in the physical properties of disperse systems would be an advantage.

The Colworth Laboratory is located on a very attractive 1200 acre country estate ten miles north of Bedford. Salaries and fringe benefits are at a level expected of a large international company and include assistance with moving expenses where appropriate.

Please write giving details of career history and quoting reference number CH-523A to:

Miss J. Kenny,
Unilever UK Central Resources Limited,
Unilever Research, Colworth Laboratory,
Colworth House, Sharnbrook,
Bedford MK44 1LQ.

3878(A)

CITY OF LONDON POLYTECHNIC
DEPARTMENT OF BIOLOGICAL SCIENCES
GRADUATE RESEARCH ASSISTANT

A graduate technical officer is required immediately to work on a World Health Organisation sponsored study of plant substances as fertility regulating agents. The project will be of interest to pharmacologists and reproductive physiologists. Experience with small mammals will be an asset. The successful applicant may be able to register for a higher degree.

Salary commencing at £3,417 including London Weighting. A full curriculum vitae giving the names and addresses of two referees should be sent to Dr J P G Williams, Department of Biological Sciences, Old Castle Street, London E1 7NT within two weeks of the appearance of this advertisement. 3864(A)

FREIE UNIVERSITÄT BERLIN
INSTITUT FÜR NEUROPSYCHOPHARMAKOLOGIE
POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for appointment from 1 February 1980 to join a research team investigating various aspects of the neurochemistry of 5-hydroxytryptamine metabolism. Experience in receptor binding would be advantageous. The post will be for two years with possibility of renewal. The salary will be according to BAT IIa (about 42,000 to 45,000 DM pa).

Applicants should write, enclosing full curriculum vitae, to: Dr. H. Rommelspacher, Institut für Neuropsychopharmakologie der Freien Universität Berlin, Ulmenallee 30, D-1000 Berlin 19, West Germany (Tel: 030-3003239). W591(A)

HARVARD UNIVERSITY
SCHOOL OF PUBLIC HEALTH
RESPIRATORY PHYSIOLOGIST

Applications are invited for the position of Assistant Professor of Respiratory Physiology. Successful candidate will join group of basic and applied scientists in the field of respiratory mechanics. Research mainly in chest wall mechanics, actions of respiratory muscles, and mechanical factors in the control of breathing in animals and humans; collaboration with groups studying lung defense mechanisms, occupational and environmental health, and adult and pediatric chest medicine. Good background in math and physics with several years' postdoctoral research experience in respiratory physiology required.

Interested applicants should forward curriculum vitae, three letters of recommendation, and selected reprints to: Jere Mead, MD, Professor, Department of Physiology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

Harvard is an Equal Opportunity/ Affirmative Action Employer. W586(A)

UNIVERSITY OF EDINBURGH
SENIOR LECTURER/ LECTURER IN PHYSIOLOGY

Tenable from 1st January 1980.

Salary on the scale £8,698 to £10,775 for Senior Lecturers, or £4,333 to £8,992 for Lecturers.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (6 copies) together with curriculum vitae and the names and addresses of three referees should be lodged not later than 8th January 1980.

Please quote ref no 519. 3871(A)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHARMACOLOGY
Applications are invited for a RESEARCH ASSOCIATE OR SENIOR RESEARCH ASSOCIATE

to work on an MRC-funded project involving analysis of stimulus-secretion coupling in pancreatic cells by laser light scattering techniques. The appointment is tenable for up to two years and salary will be in the range £5,199 to £6,932, or £6,066 to £8,892, according to qualifications and experience, with U.S.S. A biophysical background or experience in computing and photon correlation spectroscopy would be advantageous but applicants with a PhD in Physics and an interest in its biological applications would also be welcomed.

Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to, Dr E K Matthews, University Reader in Pharmacology, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD. 3873(A)

BASIC GRADE BIOCHEMIST

required for the remaining five months of a hospital grant to measure plasma catechol amines by a working radio enzyme method starting on 7.1.80. Salary on scale £4,205 — £5,714 inc.

Application forms obtainable from Department of Personnel, University College Hospital, Gower Street, WC1E 6AU. Tel: 387 9300 Ext. 381. 3904(A)

METABOLISM AND PHARMACOKINETICS

Experienced in the respiratory field? Interested in Deposition of drugs in the Lungs?

A vacancy has arisen in our Metobolic Studies Department for a person to lead a small team concerned with the deposition and absorption of medicinal aerosols in the lungs. Ideally we are interested in someone with a sound knowledge of human and animal respitory systems, both structure and function, who have an interest in Pharmacokinetics. The particular concern of the department is in the dosimetry and site of deposition of potential aerosol products but an interest in fundamental principles involved in this area will be encouraged.

Suitable candidates will probably have 1-3 years post-doctoral experience in the appropriate field of interest or have a good degree and equivalent experience.

The position carries a competitive salary which will reflect the experience of the successful candidate.

Filsons offer attractive working conditions in a pleasant part of the country. Other benefits including flexible working hours, annual bonus, an active sport and social club and assistance with relocation where appropriate.

Applicants, male or female should telephone or write for an application form to Cathy Mason, Personnel Officer, Fisons Ltd, Pharmaceutical Division, Bakewell Road, Loughborough, Leics. Tel: 0509 66361.
Quoting ref RD75/N 3903(A)

FISONS

PHARMACEUTICAL DIVISION

Public Health Laboratory Service

Communicable Disease Surveillance Centre

Technical Editor

The Communicable Disease Surveillance Centre of the Public Health Laboratory Service requires a technical editor, deputy to the medical editor, for the weekly Communicable Disease Report and other communicable disease surveillance reports. Candidates must be university science graduates with experience in the National Health Service and be fully conversant with clinical and microbiological terminology. Clinical and or microbiological experience an advantage.

Salary scale: The appointment will be on NHS terms and conditions and the salary scale will be within the range £5935 to £8083 (including London Weighting Allowance).

A job description and application form can be obtained from Mr A Collins, Centre Administrator, Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ. Tel. 01-200 6868. Closing date: 31 December 1979.

3876(A)

PHLS

UNIVERSITY OF ESSEX

POST-DOCTORAL MICROBIAL ECOLOGIST

Applications are invited for a NERC post-doctoral Fellowship, to work in the Department of Biology with Dr D B Nedwell. The project is intended to develop methods for measuring turnover of selected organic molecules in anaerobic saltmarsh sediments in order to elucidate patterns of carbon flow in the microbial community. The programme will involve field work and laboratory work on model systems. Experience with gas chromatography, radiotracers and continuous culture would be advantageous. The appointment commences as soon as possible for three years, and salary will be at the appropriate point on the Range IA scale (£4333 to £7521).

Applications (two copies) including a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to the Registrar (AG/95/N) University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained. 3875(A)

INSTITUTE OF CANCER RESEARCH

ULTRASOUND IN CANCER DIAGNOSIS

A RESEARCH TECHNICIAN (Physical sciences)

is required to join a multi-disciplinary research group working on Ultrasonic imaging and analytical methods in cancer investigation. This job, which is likely to include assisting in ultrasonic examination of patients and subsequent computer analysis of data, will be based at the Royal Marsden Hospital, Sutton, Surrey.

Candidates should have had training, and preferably some experience, in a physical science or in radiography and should have obtained HNC, BSc or an equivalent qualification in an appropriate subject.

Salary will be on MRC scales commencing at a point in the range £3,768 p.a. to £4,008 p.a. Applications with full curriculum vitae in duplicate and the names and addresses of two professional referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/88. 3902(A)

ASSOCIATESHIPS

QUEEN'S UNIVERSITY AT KINGSTON

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIATESHIP

Applications are invited for a post-doctoral research appointment involving the development of solid state hydrogen ion conductors and the physics of fast ion transport. The appointment is for an initial period of one year renewable for a second year.

Salary $14,500 — $15,000 depending upon qualifications. Please apply including the names of two referees to: Dr M Sayer, Department of Physics, Queen's University, Kingston, Ont., Canada K7L 3N6 W600(O)

nature

London
3 Dyers Buildings
London EC1N 2NR
01-831 6901

New York
Suite 832
50 Rockefeller Plaza
New York, NY 10020
(212) 765-5758

CONFERENCES

CONFERENCE RECEPTOR MEDIATED BINDING AND INTERNALIZATION OF TOXINS AND HORMONES

Conference sponsored by US Army Medical Research Institute for Infectious Diseases to be held at Frederick, Maryland on March 24-26, 1980. Program composed of talks by 25 international scientists expert in the areas of toxins and polypeptide hormones. Attendance will be limited by space to 130 scientists.

Further information may be obtained and application made by writing to Dr John L. Middlebrook, Pathology Division, USAMRIID, Fort Detrick, Frederick, Maryland 21701. W601(C)

ASSISTANTSHIPS

UNIVERSITY OF CALGARY

GRADUATE ASSISTANTSHIPS (Teaching and Research)

Available for students proceeding to the MSc and PhD degrees in all branches of Chemistry and Biochemistry. The assistantships are valued at $7,400 to $9,000 p.a. and include remission of program fees. Travel allowance also available.

For further information write to Department of Chemistry, Chairman of Graduate Studies, University of Calgary, Calgary, Alberta, Canada T2N 1N4. Please indicate field of interest. W579(P)

GRANTS and SCHOLARSHIPS

THE UNIVERSITY OF WESTERN AUSTRALIA Perth FORESTS DEPARTMENT POSTGRADUATE SCHOLARSHIP IN BOTANY

Applications for a Forests Department Postgraduate Scholarship in Botany are invited from graduates with a first-class upper division second-class honours degree in Botany, and Forestry or Agriculture graduates whose courses of study have extended over at least four years and who have had appropriate research experience. The Scholarships, financed by the Forests Department of Western Australia, will be tenable for up to three years. The value of the Scholarship is $4000 per annum. The student will be required to undertake research in a field covering aspects of physiology, ecology or mycology in relation to multiple use forestry, or such other fields as may be prescribed. Applicants are advised to obtain further information on possible fields of interest by writing to the Head of the Department of Botany.

Applications in triplicate stating personal particulars, academic record, research experience, etcetera should reach the Registrar, University of Western Australia, Nedlands, 6009, Western Australia, by *31st December, 19*. Candidates should request two academic referees to write immediately to the Registrar. 3889(H)

EMBO COURSES PROVISIONAL PROGRAMME 1980

Subject	Organizer and Address for Further Information		Date Place Number of Participants
PRACTICAL COURSES			
Molecular Biology of Membranes	Professor G. Schatz	Biozentrum der Universität Klingelbergstraße 70 4056 Basel SWITZERLAND	14-27 September Basel 25
Immunocytochemistry and its Application in Brain Research	Professor D.F. Swaab	Netherlands Institute for Brain Research P.O. Box 41850 1009 DB Amsterdam THE NETHERLANDS	27-30 May Amsterdam 15
Teratomas	Dr. M.L. Hooper	Institute of Genetics Church Street Glasgow G11 5JS SCOTLAND	17-29 March Glasgow 20
Bacterial Genetics	Professor P. Starlinger	Institut für Genetik Weyertal 121 5 Köln 41 FEDERAL REPUBLIC OF GERMANY	8-27 September Köln 22
Antibodies as Probes in Developmental Biology	Dr A.E. Wild	Department of Biology Medical & Biological Sciences Building Bassett Crescent East Southampton SO9 3TU ENGLAND	7-18 July Southampton 15-20
The Molecular and Cell Biology of Trypanosomes	Dr. F.R. Opperdoes	Research Unit for Tropical Diseases International Institute of Cellular and Molecular Pathology Av. Hippocrate, 74 1200 Brussels BELGIUM	18-29 August Brussels 20
Electron Microscopy of Nucleic Acids	Dr. J. Meyer	Biozentrum der Universität Abteilung Mikrobiologie Klingelbergstraße 70 4056 Basel SWITZERLAND	18-23 August Basel 12
Hybridomas	Dr. G. Köhler	Basel Institute for Immunology 487 Grenzacherstraße 4005 Basel 5 SWITZERLAND	7-18 July Basel 18
3-Dimensional Structure Analysis by Electron Microscopy	Dr. L.A. Amos	MRC Laboratory of Molecular Biology Hills Road Cambridge CB2 2QH ENGLAND	20-26 September 20
Glycoconjugates	Professor J. Montreuil	Université des Sciences et Techniques de Lille I Laboratoire de Chimie Biologique et Laboratoire Associé au Centre National de la Recherche Scientifique no. 217 59655 Villeneuve d'Ascq Cédex FRANCE	8-20 September Villeneuve d'Ascq 20
Cosmid Cloning Course	Dr. B. Hohn	Friedrich Miescher Institut P.O. Box 273 P.O. Box 273 4002 Basel SWITZERLAND	8 days in September Basel 12-15
An Advanced Course in Molecular Genetics; Plasmid Structure and Function	Dr. J. Collins	Gesellschaft für Biotechnologische Forschung mbH Mascheroder Weg 1 33 Braunschweig-Stöckheim FEDERAL REPUBLIC OF GERMANY	21 July – 6 August Braunschweig 20
Molecular Approaches to Study Cell Differentiation	Dr. P.R. Harrison	Beatson Institute for Cancer Research Garscube Estate Switchback Road Bearsden Glasgow G61 1BD SCOTLAND	14-27 September Glasgow 20-25
Recombinant DNA Technology	Dr. F. Blasi	Centro di Endocrinologia e Oncologia Sperimentale del CNR Istituto di Patologia Generale University of Naples Via Sergio Pansini 5 80131 Naples ITALY	29 September – 13 October Naples 16
Molecular and General Genetics of Yeast	Dr. A. Hinnen	Friedrich Miescher Institut Postfach 273 4002 Basel SWITZERLAND	18 August – 5 September Basel 15
Membrane Biogenesis	Dr. H. Garoff	European Molecular Biology Laboratory Meyerhofstraße 1 69 Heidelberg FEDERAL REPUBLIC OF GERMANY	5-16 October Heidelberg 30-36
Cellular Immunology: A Practical Course in Methods	Professor H. Wigzell	Department of Immunology Biomedical Centre University of Uppsala Box 582, BMC 75123 Uppsala SWEDEN	6-19 July Uppsala 16
Ultrastructural Analysis of Nuclear and Organelle Genetic Material	Dr. E.L. Benedetti	Laboratoire de Microscopie Electronique I R B M, CNRS 2 place Jussieu 75221 Paris Cédex 05 FRANCE	3 weeks in March or April Paris 15-18
Electron Microscopy of Nucleic Acids	Dr. H. Delius	European Molecular Biology Laboratory Meyerhofstraße 1 69 Heidelberg FEDERAL REPUBLIC OF GERMANY	21-29 October Heidelberg 8
DNA Sequencing Methods	Dr. G. Bernardi	Institut de Recherche en Biologie Moléculaire du CNRS Université Paris VII – Tour 43 2, place Jussieu 75221 Paris Cédex 05 FRANCE	to be decided Paris 16
LECTURE COURSES			
Genome Organization and Function	Professor H.G. Zachau	Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität Goethestraße 33 8 München 2 FEDERAL REPUBLIC OF GERMANY	31 August – 13 September Spetsai
Molecular and Cellular Aspects of Early Mammalian Development	Dr. W.D. Billington	Department of Pathology University of Bristol Medical School University Walk Bristol BS8 1TD ENGLAND	21-28 September Gonia Chania (Crete) 80-100
Chromosome Damage and Repair	Professor K. Kleppe	Department of Biochemistry University of Bergen Arstadveien 19 5000 Bergen NORWAY	27 May – 5 June Bergen 80
Basic Lecture Course of Molecular Genetics: Mechanisms of Chromosome Replication, Recombination and Repair	Professor A. Falaschi	Laboratorio di Genetica Biochimica ed Evoluzionistica Via S. Epifanio, 14 27100 Pavia ITALY	2nd half of September Pavia Pavia 50-60

W581(D)

COURSES

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1980 NEUROBIOLOGY COURSES & WORKSHOPS

The Neurobiology of Behavior, June 6-June 20
Instr.: E Kandel, J Koester, College of Physicians & Surgeons, Columbia University; K Pearson, University of Alberta

Nervous System of the Leech, June 9-June 29
Instr.: J Nicholls, Stanford University Medical School; K Muller, Carnegie Institution of Washington; I Parnas, Hebrew University; B Zipser, Cold Spring Harbor Laboratory

Mammalian Central Nervous System, June 9-July 2
Instr.: D Hubel, H Sherk, Harvard Medical School; P Kirkwood, Institute of Neurology, London; J Malpeli, University of Illinois; W Rymer, Northwestern University Medical School

The Synapse: Cellular & Molecular Neurobiology, June 22-July 12
Instr.: R Rahamimoff, Hebrew University Medical School; U J McMahan, Stanford University; B Katz, University College London; C Stevens, Yale University; D Yoshikami, University of Utah

Basic Neuroanatomical Methods, July 8-July 28
Instr.: E G Jones, B K Hartman, Washington University; P J Hand, University of Pennsylvania

Principles of Neural Development, July 15-July 29
Instr.: D Purves, Washington University School of Medicine; P H Patterson, Harvard Medical School

Electrophysiological Methods for Cellular Neurobiology, July 21-August 10
Instr.: J Kehoe, R Kado, Ecole Normale Supérieure; D Chiarandini, New York University School of Medicine; E Stefani, Instituto Politecnico Nacional

Registration Deadline: March 15, 1980. For applications write to:
Registrar, Cold Spring Harbor Laboratory
Box 100, Cold Spring Harbor, New York 11724
preferably by February 1. W597(D)

CSH

EUROPEAN MOLECULAR BIOLOGY ORGANISATION
INTERNATIONAL WORKSHOP
on
Immunological Aspects of Nuclear Components
Portofino, Italy, April 14-16 1980

Leonardo Santi (Genova) and Giorgio Vidali (New York) Organizers
Michael Bustin (Bethesda) and Franco Celada (Genova) Scientific Committee

The invited speakers will include: H. Zachau (Munchen), M. Bustin (Bethesda) D. Stollar (Boston), M. Romani (New York), R. Gallo (Bethesda), H. Bautz (Heidelberg), J. Sommerville (Fife), T. Martin (Chicago), B.E. Erlanger (New York), H. Kunkel (New York), R. Lahita (New York), E.M. Tan (Denver), M. Reichlin (Buffalo), P.H. Lambert (Geneve), A. Marmont (Genova), S. Bombardieri (Pisa), G. Klein (Stockholm), R. Baserga (Philadelphia), L. Philipson (Uppsala), J.S. Pagano (Chapel Hill), L. Santi (Genova), M. Van Regenmortel (Strasbourg), M. Leng (Orleans), L. Zardi (Genova) M. Poirier (Bethesda).

The total number of participants will be limited to 50 and applications from young investigators are encouraged. Further details from Dr. Leonardo Santi, Instituto di Oncologia, Viale Benedetto XV 10, 16132 Genova, Italy and from Dr. Giorgio Vidali, The Rockefeller University, New York, N.Y. 10021, USA. Closing date for applications February 15, 1980. W587(D)

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September 1980 to September 1981 (1982*).

Applicants should normally have a good degree in Physiology or be medically qualified.

Further details and application form from: Dr S Rowlands, King's College, Strand, London SC2R 2LS.

Closing date for applications: 31 March 1980. 3882(D)

FELLOWSHIPS

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Notice is hereby given that an election of Junior Beit Fellows to begin work on 1 October 1980 will take place in May 1980. Junior Fellowships carry an initial value of £4,333 to £5,199 plus £740 London Allowance, plus yearly increments for three years. There will be the usual university superannuation contributions and benefits. Candidates must have taken a degree in a faculty of a university approved by the Trustees in Her Majesty's Dominions, Protectorates and Mandated Territories, India, Pakistan, the Republic of Ireland and the United Kingdom, or a medical diploma registrable in the UK. Elections to Junior Fellowships are rarely made above the age of 35 years. Applications from candidates must submit evidence where they propose to work, which must be in Great Britain or Ireland.

Forms of application and all information may be obtained from Professor W G Spector, (Secretary), Pathology Department, St Bartholomew's Hospital, London EC1A 7BE. For Overseas candidates forms of application may be obtained from, The Secretary, South African Medical Council, PO Box 205, Pretoria, South Africa; The Ministry of Health, The Government of India, New Delhi, India; The Secretary, Department of Education and Science, PO Box 826, Canberra City, A C T 2601, Australia; The Department of Health, Wellington, New Zealand; and, The Canadian Medical Association, 1867 Alta Vista Drive, Ottawa 8, Ontario, Canada. 3863(E)

THE ROYAL SOCIETY
E.P.A. Cephalosporin Fund SENIOR RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for the E.P.A. Cephalosporin Fund Senior Research Fellowship, endowed by the Trustees of the E.P.A. Cephalosporin Fund to provide a research fellowship in the medical, biological and chemical sciences. On this occasion applications are restricted to those working in the field of biological chemistry. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment, which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from 1 October 1980 (or another date to be arranged) and may be renewable for one further five-year period. Applicants should preferably be under 40 on 1 October 1980. The stipend scale will be £9138 × £300 to £10 518 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, and returned by 15 January 1980. 3859(E)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL
POST-DOCTORAL FELLOWSHIP (MRC) IN HUMAN PHYSIOLOGY

A new group has been established in the Department of Physiology and Pharmacology under the leadership of Dr C T M Davies (MRC) to investigate the physiology and biochemistry of human muscle in the elderly.

Postgraduates with appropriate experience of physiology interested in this Fellowship should write in the first instance to Professor P H Fentem, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, enclosing a curriculum vitae and the names of two referees. 3869(E)

FOULKES FOUNDATION FELLOWSHIPS 1980

The Foulkes Foundation provides financial support for recently qualified science graduates with research experience who want to study medicine and for medical graduates who want to take a science degree. In both cases graduates must intend to do medical research after qualification.

Application forms and further information are available from: The Administrative Assistant, Foulkes Foundation Fellowships, The Ciba Foundation, 41 Portland Place, London W1N 4BN, enclosing a s.a.e. Closing date 31 March 1980. 3867(E)

UNIVERSITY OF NOTTINGHAM SCHOOL OF AGRICULTURE
DEPARTMENT OF PHYSIOLOGY & ENVIRONMENTAL STUDIES
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Application forms and further details can be obtained from The Secretary, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. 3905(E)

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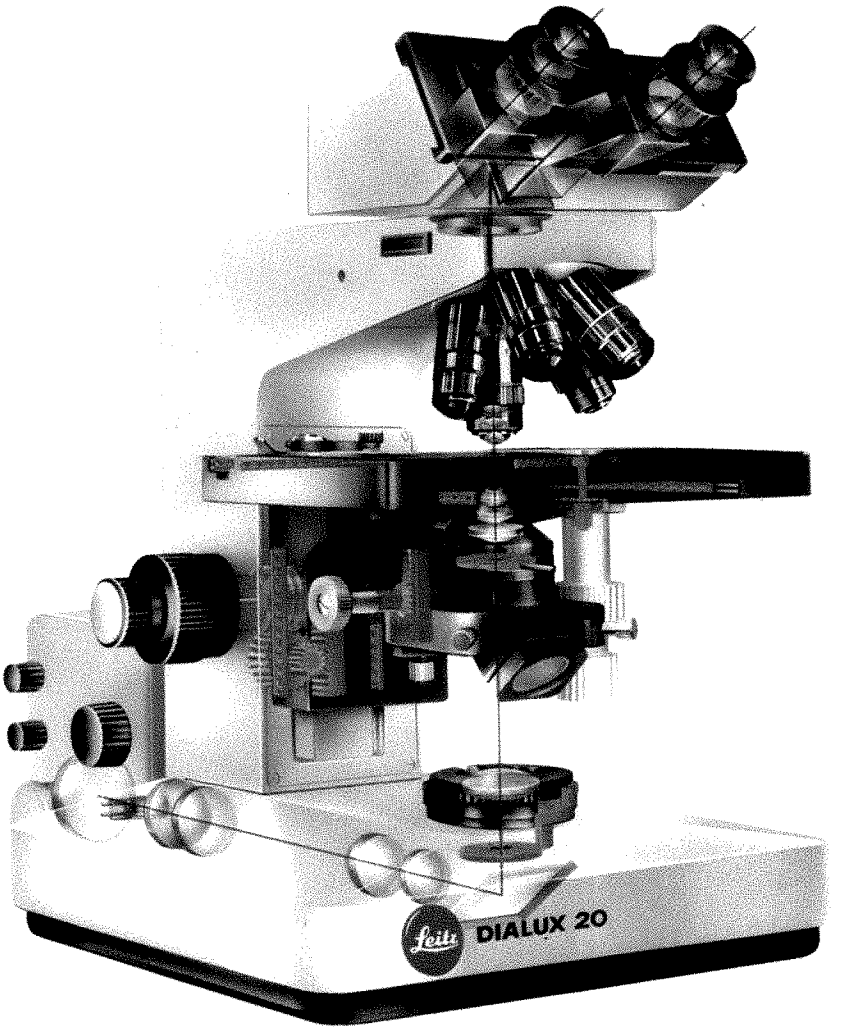
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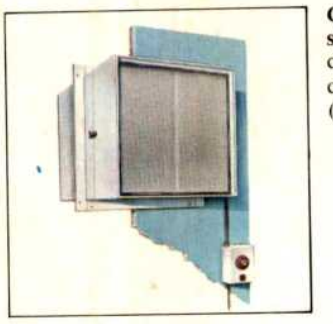
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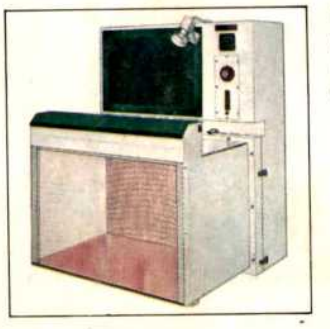
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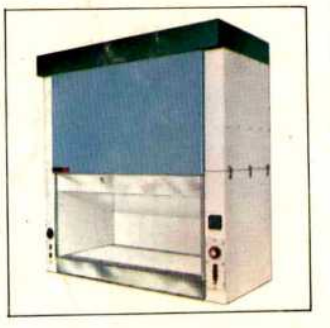
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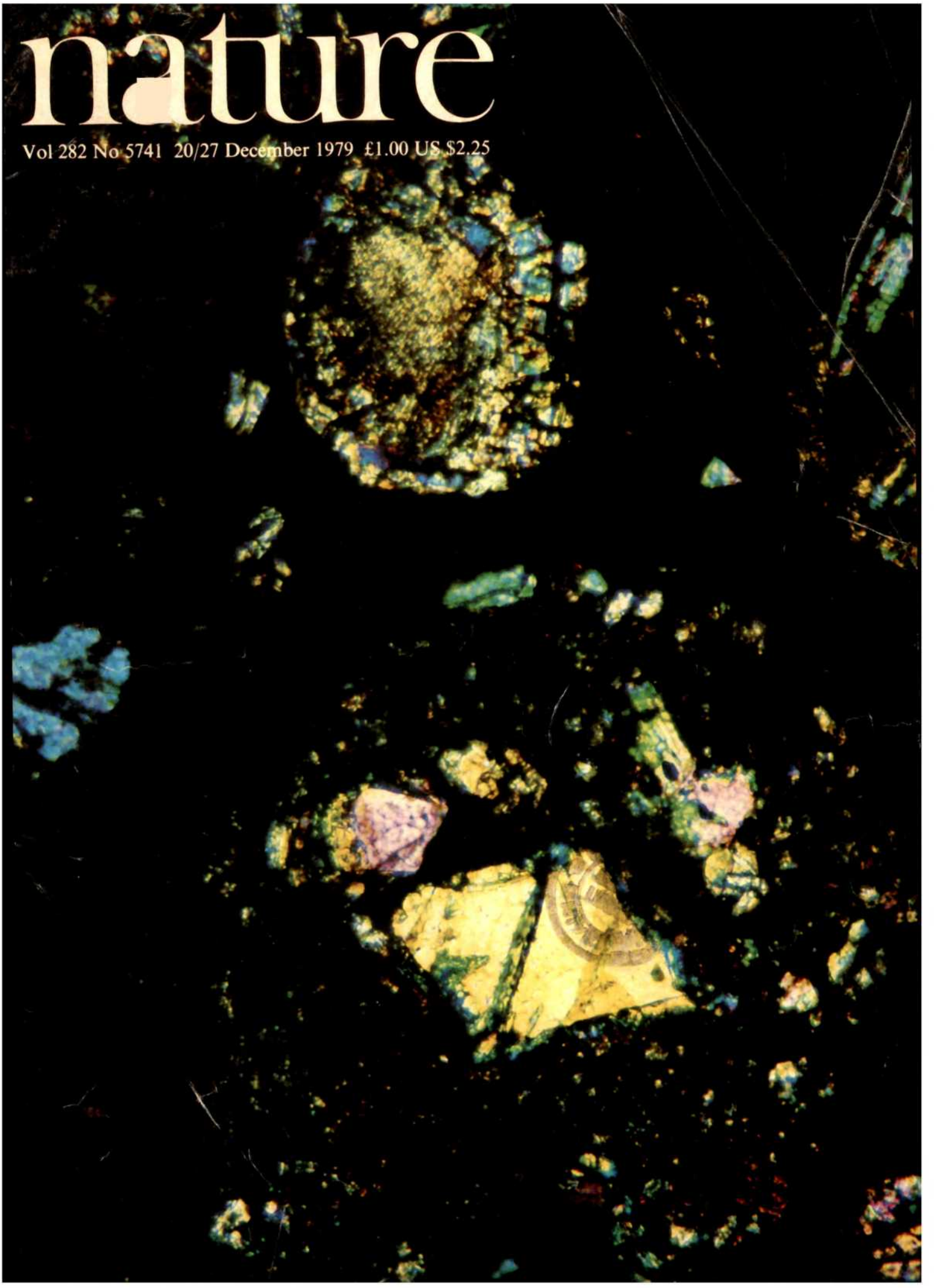
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Chainpur meteorite in thin section showing chondrules set in dark matrix. Transmitted light with crossed polars. Yellow diamond-shaped crystal (long axis 0.4 mm) is olivine. Studies of meteorites appear on pages 785, 790 and 827.

Photo: Courtesy British Museum (Natural History)

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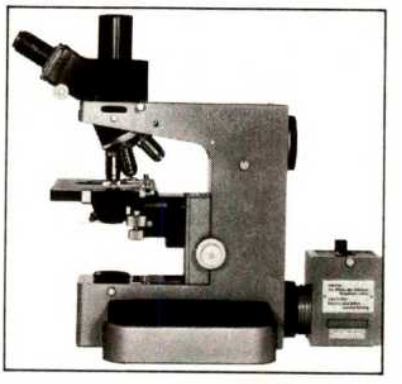
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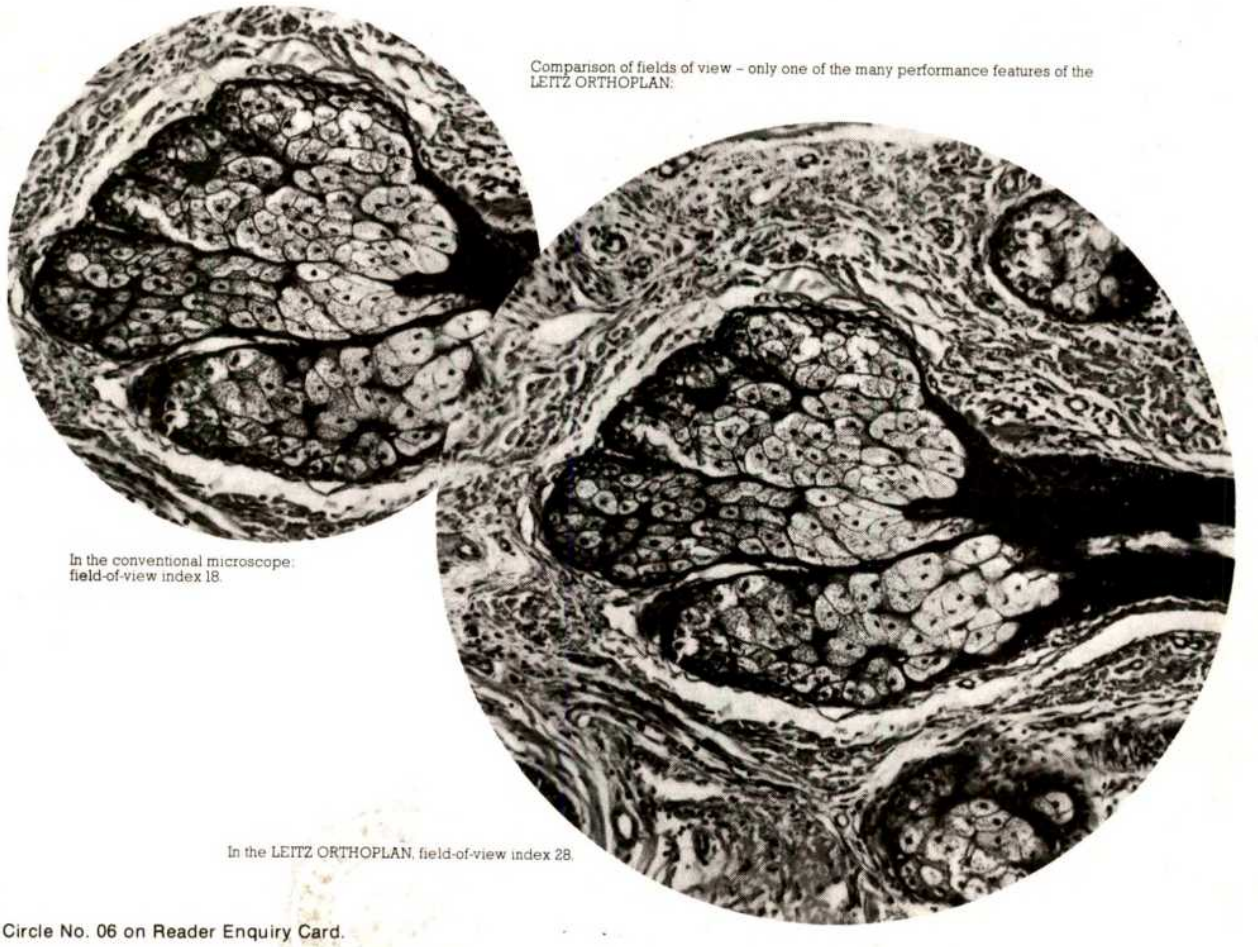
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Abbreviations should follow the *World List of Scientific Periodicals*, fourth edn (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

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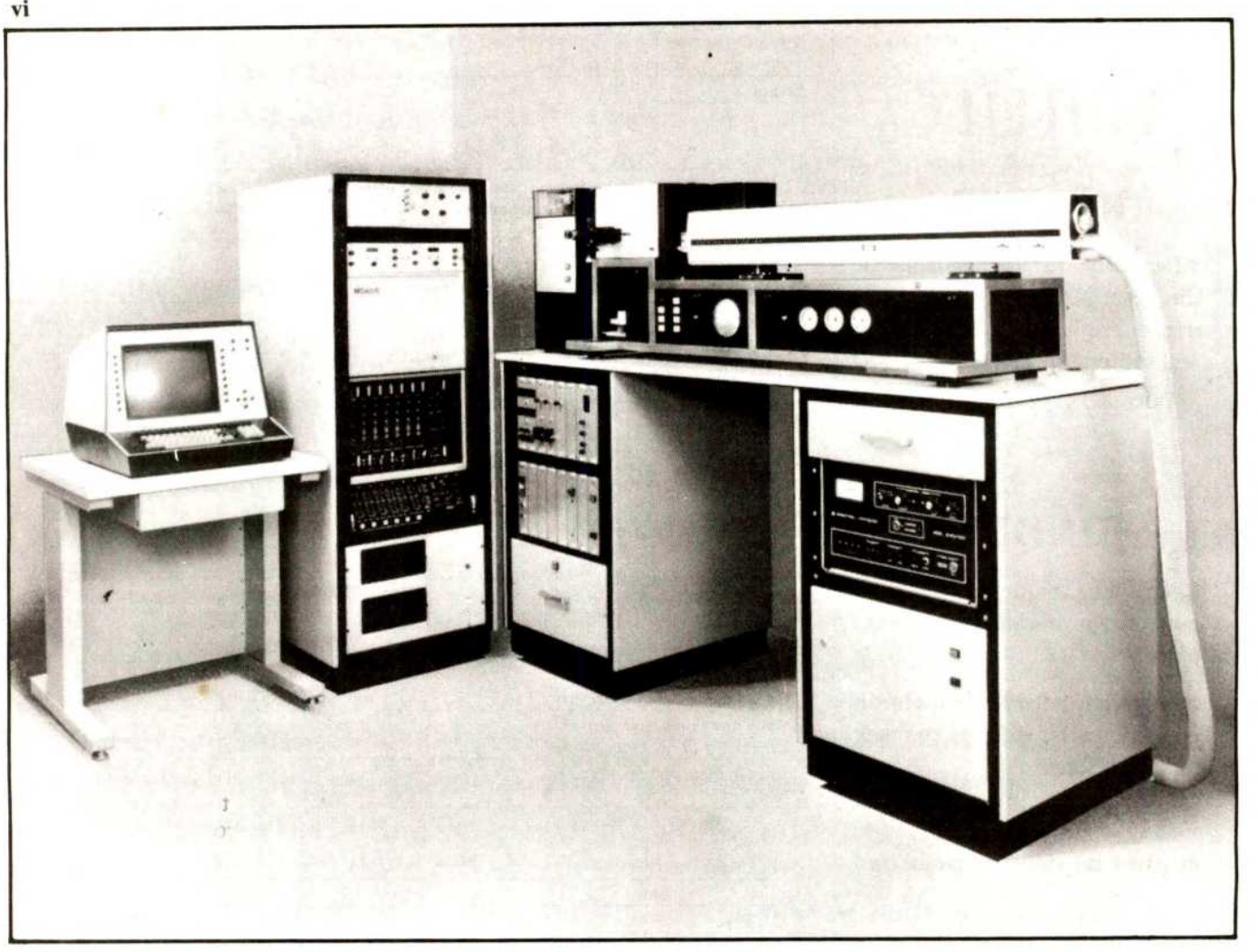
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The authorship of the article 'Crust of Oceanic Affinity in Iceland', *Nature* **281**, 347 (4 October) emerged incorrectly as a result of a misunderstanding between us and our typesetters on the telephone.
It should read:
Scientific Party, Iceland Research Drilling Project
(J.L. Gibson, editor)



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20-27 December 1979

Into the 1980s

CHANGES of Editor at *Nature* are relatively rare: my departure at the end of 1979 will only be the fifth such change in 110 years (admittedly the figures are somewhat distorted by the extraordinary 50 years which the first editor, Sir Norman Lockyer, served). A new editor has yet to be appointed; for the interim Peter Newmark, at present Deputy Editor, will take over. Dr Newmark has had overall responsibility for the selection of biological manuscripts for almost six years and has done this job with distinction. *Nature* will be in excellent hands.

The production of the journal, week by week, year by year, is a collaborative enterprise involving a wide variety of people. It requires the continuing support and encouragement of the board of directors; it requires the endless attention to detail of typesetters, printers and blockmakers; it requires the enthusiasm of advertising, promotion and circulation departments; it requires a lot of hard work from our secretarial and artwork teams. For this I have good cause to be very grateful. And yet my most profound thanks must go to the two groups of people, one small, one large, which between them ensure the continuing vitality of the journal: the editorial staff and a small army of outside helpers.

Tributes to staff can so easily be perfunctory; this one is very heartfelt. My fifteen colleagues who between them decide what goes into the journal, when and how, are a quite outstanding and thoroughly professional team, whose clear appreciation of what *Nature* ought to be and how *Nature* ought to change has been a constant support to me. Maintaining *Nature's* standards calls for a particularly high degree of dedication to work which can at times be exceptionally onerous. The editorial team possesses this dedication in great measure.

And yet all the in-house ability would count for nothing if we could not depend on scientists from all disciplines and all parts of the world for willing help. One of the interesting things about *Nature*, is that this week's reader may be next week's author, adviser, referee or correspondent. The journal belongs more to a community than to a company, an editor or an editorial team. And without the continued encouragement, help and criticism we get from the scientific community, the journal could not survive. We thank you, the scientist, even though occasionally we choose to ignore your advice or refuse to publish your contribution!

ALL manner of scientific and science-political themes rise and fall during an editor's tenure, and perhaps it is foolish to single one out for a last comment. After all reams could be written about the growth of public participation in science-policymaking, the problems of a career structure for scientists, the growing awareness of the vulnerability of the scientist to human rights violations, the place of scientists in development, risks to science funding in a weak economy and so on. Yet if there is one issue which consistently fails to capture headlines yet which ought to interest the scientific community, it is that of arms control and disarmament.

The 1970s has not been a hopeful decade. SALT 2, still not ratified, took an extraordinary time to negotiate and, as far as one can see, does little but confirm existing levels. And in order to sell SALT to the American people, other vigorous armaments programmes are promised. A comprehensive test ban treaty, on which hopes were raised recently, is now unlikely to appear in the near future; if ever it does emerge it is going to be so circumscribed as to be meaningless. The Non-Proliferation Treaty, due for review in 1980, has acquired few new signatories whilst a novel brand of proliferation has emerged, as practised by Israel, South Africa and Pakistan — proliferation by assumption. Multilateral Balanced Force Reduction talks in Vienna are hopelessly bogged down.

There is little progress towards a chemical weapons treaty, whilst there are growing fears of chemical re-armament. Meanwhile arms sales to the developing world continue with the utmost vigour, and nuclear nations improve their arsenals not so much quantitatively but qualitatively. And new weapons, such as laser and particle-beam devices against ballistic missiles get expanded research budgets, regardless of the enormous difficulties there will be in converting ideas into hardware.

Scientists should have a lot to say, and publicly, about the technological arms race. After all in most countries ministries of defence are the nation's largest single employers of scientists and technologists. And yet in recent years the enthusiasm that there once was for asking pointed questions on the assumptions behind military expenditure has largely dissipated — no doubt because of the thankless nature of the task and the seeming impossibility of deflecting inexorable trends.

Will any measures of real self-restraint emerge in SALT 3? Are nuclear weapons bound to spread indefinitely? Is there no way of diverting the resources devoted to military purposes into more constructive channels? These are questions for the 1980s . . . and beyond.

David Davies.

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Nature Vol. 282 20-27 December 1979

Energy shifts meet environmentalist protests

Moves to convert power stations from oil to coal are generating controversy about the effects on air quality. **David Dickson** reports on one such dispute in New York City.

In a classic trade-off between energy and environmental concerns, New York city last week approved a temporary waiver of its air pollution controls to allow a local power company, Consolidated Edison to burn fuel with a greater sulphur content than is currently permitted.

The company hopes to be able to demonstrate that it is able to use high rather than low sulphur content fuels at its power plants in the city without exceeding federal air pollution standards, and that this will make it possible for the plants to convert from oil to coal, a move supported by President Carter as part of his strategy to reduce dependence on foreign oil.

Local environmental and community groups, however, are protesting against the proposal, which has yet to be given official approval by the federal Environmental Protection Agency. They argue that current controls permitting only the burning of oil with a low sulphur content have been one of the most effective anti-pollution measures in the city's history — and that moving back to higher sulphur content fuels could eliminate some of the gains of the past decade.

Con. Ed. has for several years been trying to get permission to increase the sulphur content of the fuel burnt in its power plants. It claims that not only would conversion to coal be in line with administration policy, but that with escalating oil prices, it would also be financially beneficial to customer companies.

In the previous year, the company's application has been turned down, largely on the grounds that the company has not been able to demonstrate that the current policy results in any particular hardship. Since then, however, the political climate for such decisions has changed dramatically.

In his energy message to Congress in July, for example, President Carter stressed his support for power companies converting from oil to coal. He is expected to submit to Congress soon measures costing even more than the $5 billion that he originally proposed to stimulate the use of coal.

A number of utilities have already benefited from the new climate. In Massachusetts, for example, the New England Power Company, after a four-year battle against stiff local opposition, recently obtained permission to convert three out of four units of a 1,650 megawatt plant from oil to coal, claiming that this would reduce oil consumption in the region by 2.5%.

Last year the company was given permission for an experimental period to burn oil with double the previously permitted 1.1% sulphur content. Company officials admit that pollution levels increased as a result — but point out that they were still within federally-permitted levels.

The current application form Con. Ed. would allow the company to increase the sulphur content of its fuel from 0.3% to 1.5%. It claims that converting three power plants from oil to coal would reduce the nation's dependence on oil by 15 million barrels a year — and would also reduce its customer's bills by 150 million.

"The reason for requesting temporary permission to burn high sulphur content oil is to demonstrate the validity of our contention that such a move will not violate federal primary air quality standards", a spokesman for the company said last week.

Permission for a temporary waiver from current controls has already been given by the New York State Department of Environmental Conservation, and by the New York State Department of Environmental Protection. The application will soon be published for public comment by the EPA, and a final decision is expected by next March or April.

Con. Ed.'s application is being strongly resisted by environmental and community groups in New York. "The City's current requirements that only clean fuels — that is low sulphur content oil and natural gas — can be used has been one of the most successful pollution control strategies that the city has had, and the sulphur content in the air has dropped by 40% since the controls were introduced in 1972", says Marcy Benstock, Director of the New York Clean Air Campaign.

Ms Benstock claims that a comparable reduction in dependence on foreign oil could be achieved through an intensive energy conservation programme. She quotes evidence from a report by researchers at Harvard University that the US could consume 30% to 40% less energy than at present and still enjoy the same standard of living.

Furthermore, she claims, the apparent reduction in costs could be eaten away by additional medical bills and other damage caused by increasing pollutant levels. Even pollution control devices, she argues, will not be able to remove all the additional pollutants caused by converting to coal, such as some of the toxic trace elements.

A new "wrinkle" as one EPA official puts it, recently to have entered the debate, is concern over the extent to which increased sulphur emissions could contribute to a problem receiving growing attention in the US, namely acid rain.



Smog in New York city: will Carter's energy policy make it worse?

According to a spokesman for Con. Ed., the company does not consider this to be too much of a problem since "by and large the sulphur emissions go out to sea". Others, however, argue that not enough is yet known about transportation patterns to judge the potential degree of the problem.

"At present the burden tends to be on society to show that such things are harmful. But the alternative is equally rational, namely that increased emissions should not be allowed until it can be clearly shown that they are not harmful", says Dr Ellis B Cowling, a plant pathologist from North Carolina State University who has studied the acid rain problem, and gave evidence at an earlier hearing on Con. Ed.'s request.

The transport of pollutants is one of the subjects to be examined in a regional study of metropolitan New York and the adjacent areas of New Jersey and Connecticut that has just been announced by the National Commission on Air Quality.

The company's application to use higher sulphur content fuels is being opposed not only by local groups such as the New York statewide Senior Action Council, and People Outraged With Energy Rates (POWER), but also national groups such as the Sierra Club and the Natural Resources Defense Council.

So far, however, their protests have made little impact, and with the current crisis in Iran bolstering programmes for domestic energy prodution, as well as an administration keen to help power companies comply with federal legislation in the least burdensome way — and a relative lack of financial and human resources compared to those seeking the application — their chances of stopping Con. Ed.'s plans seems increasingly slim. □

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UN agrees on science and development centre

AFTER three weeks of intense debate, member nations of the United Nations agreed late last week to set up a new policy unit to help carry out the recommendations of the Conference on Science and Technology for Development (UNCSTD) held in Vienna in August.

The new unit will be known as the Centre for Science and Technology for Development. It will be headed by an Assistant Secretary General, and is expected to involve between eight and ten new staff appointments, as well as absorbing some of the resources of the present Office of Science and Technology, which is to be abolished.

The main function of the new centre will be to service the Intergovernmental Committee which, it was agreed at Vienna, should be set up to attempt to coordinate the various programmes within the UN system concerned with applying science and technology to development, and to stimulate further activities in this direction.

A resolution calling for the setting up of the centre was finally agreed in the early hours of last Friday morning by the second committee of the UN general assembly, currently meeting in New York.

In their final form, the institutional arrangements are broadly in line with proposals made by the Group of 77, on behalf of the developing countries, that a strict interpretation of the agreement reached in Vienna implied the creation of a new secretariat.

A number of industrialised countries, and in particular the member countries of the European Economic Community, argued strongly that no new institutional arrangements should be made — and that any new functions could be carried out by existing machinery under the Department of International Economic and Social Affairs.

In the end, however, it proved impossible to devise a compromise formula which all sides could agree upon. And the decision to establish the centre — which will report to, but not be under the direct control of, the Director General for Development and International Economic cooperation, Mr Kenneth Dadzie — was put to the vote.

The result was 97 votes in favour, none against, and 20 abstentions. Countries abstaining include members of the EEC and the countries from the eastern European bloc. Those who voted in favour included not only the developing nation members, but also the Untied States, Canada, and the Scandanavian countries.

The outcome had been held up pending the resolution of a dispute over how much direct influence UN member states should have over the way in which the United Nations Development Programme disburses the "interim fund" which the Vienna conference agreed should be set up prior to the establishment of a long-term science and technology financing system.

The Group of 77 submitted a document arguing that there should be direct involvement of the IGC — which is open to all UN member states — in the administration of the fund. The developed countries, however, and in particular the US which is likely to be a major contributor, made it clear that such a decision would prejudice their agreement to contribute.

The developing countries agreed to back down on their demands, and responsibility for its management will lie in the hands of the administrator and the governing council of the UNDP, although operating within broad policy guidelines laid down by the IGC.

(A pledging conference for the interim fund will be held early next year, and although it seems that the $250 million target agreed in Vienna will not be reached, UN officials are optimistic that voluntary contributions totalling about $100 million can be raised for the two years 1980–1981.)

State Department officials in Washington later expressed satisfaction with the final outcome of the second committee's discussions, claiming that a new centre headed by an assistant director general (some developing countries had argued for an under secretary general) was "just right" — and that to have accepted the Group of 77's proposals for the administration of the interim fund would have destroyed many of the gains made in Vienna.

How the US should pursue the outcome of UNCSTD is likely to be one topic discussed by a new advisory panel on science and technology for development which is being set up under the chairmanship of Mr Tom Pickering, assistant secretary responsible for the Bureau of Oceans, Environment and Scientific Affairs.

The panel is being established under the aegis of the bureau's advisory committee, and its first meeting is likely to be held in public. Additional topics that have been proposed for discussion include energy and development, and a forward look to the global picture in the year 2000.

Meanwhile administration officals are still trying to salvage plans for an Institute for Scientic and Technological Cooperation (ISTC) a centrepiece to US presentations in Vienna, but for which funds have been refused by the Senate.

The House of Representatives has agreed that funds for the institute should be provided. But so far a joint House/Senate conference, which is bogged down on a number of foreign aid issues, has failed to agreeion whether funding for the institute should be provided.

One proposal currently being studied is that the ISTC should not, as initally envisaged by the administration, exist as a separate body within the US aid programme, but remain under the administration of the Agency for International Development, many of whose research programmes the ISTC would be taking over. **David Dickson**

US Congress approves 13% growth in military support for basic science

THE US Congress last week agreed to increase military support for basic research by 13% in the fiscal year 1980, the Senate in particular expressing its support for the Department of Defense's efforts "to overcome years of real decline in funding for this type of research".

The increase, which will bring support of basic research by DOD to about $500 million a year, is less than the 16.7% growth initially requested by President Carter. However, in line with changing social priorities, it is considerably higher than the increases in support for basic science by other federal agencies, for example, the 8% growth for the National Institutes of Health.

Overall, Congress agreed to an 8.5% growth in the Defense Department's spending on "research, development, testing and evaluation", from $12.3 billion to $13.4 billion. Much of this increase will be allocated to support of the "technology base", which includes research on high energy systems, such as lasers and particle beams, as well as high speed integrated circuits.

In particular, support for R,D,T and E activities within the defense agencies — responsible, for example, for the Defense Advanced Research Projects Agency (DARPA) — will grow by 8%.

University research workers are likely to be a beneficiary of the increased spending. Although last year Congress refused to establish a proposed "defense science and engineering programme" aimed at increasing the effectiveness of relationships between the DOD and university scientists, it has given encouragement to the department's efforts in this direction.

The Senate in particular has given support to an increased emphasis on basic research within the R and D budget. Although the House of Representatives had made several cuts in the President's budget request, many of these were restored by the Senate, and subsequently accepted at a conference between the two houses. □

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Italy threatens to disrupt three key European research institutions

Italy has drafted a career diplomat to its Ministry of Science expressly to improve its position in European research organisations. **Robert Walgate** reports from Rome.

ITALY was last week preparing to "dissociate" itself from CERN, the European organisation for nuclear research; to block (for a second year) the budget of the European Space Agency; and to refuse to fund JET, the Joint European Torus for fusion research under construction at Culham, England, for a further four-year term.

The three questions were to be raised at meetings of the council of ESA on 18 December, CERN on 19 December, and the Council of Research Ministers of the EEC on 20 December. So Vito Scalia, Italy's Minister for Scientific Research, told *Nature* in an exclusive interview last week.

At CERN, said Scalia, Italy would "consider very seriously" adopting merely an observer status if the appointment of a new director-general were not postponed until April, and if there was not "satisfactory clarifaction of the future programme of CERN".

Italy is undoubtedly the most concerned of all countries at Germany's precise intentions towards CERN, since it emerged that Germany may build a 6-km electron-position accelerator — HERA — at Hamburg. HERA is felt by some to be a competitor for CERN, which hopes to build a similar (but bigger) ring in the 1990s — LEP. Germany denies that LEP and HERA are competitive (see *Nature* 6 December p 546).

But since Germany pays the ceiling of 25% of the CERN budget, and CERN costs and salaries are high, it would arguably be cheaper for Germany to build its own LEP on home territory; hence Italy claims its fears may not be groundless.

Italy's view is also complicated by the fact that of the two principal candidates for the director-generalship of CERN one is the German director of the laboratory where HERA may be built — Professor H. Schopper — and the other is the leading Italian physicist Professor A Zichichi. Schopper's appointment at CERN "would be rather like the entry of a Trojan horse", said one Italian official. "Someone should be appointed whose Europeanism is not in question".

In contrast to this view, however, Professor Schopper has always insisted that his laboratory at Hamburg be considered an international institution. At PETRA, presently its largest accelerator and a world leader, he refused to admit groups composed of one nationality. This at one stage excluded Italy, which wanted to run a massive prestige single nation experiment at PETRA. At present there is no Italian group at PETRA, despite the fact that less than half PETRA's experimenters are German. German-Italian relations are thus somewhat tender on this issue.

At the European Space Agency, Italy will re-affirm its year-old decision to block the ESA budget because, says Scalia, "there is insufficient industrial return to compensate for our £10 million losses over two years on Spacelab". Here Italy believes that ESA's professed policy of distributing contracts among members in proportion to their contributions is manifestly not working. "Germany underwrote Spacelab 54%, Italy 18%", said a ministry spokesman. "But Germany got 62% of the contractors, ourselves 10%".

Scalia also criticised France and Germany for failing to contribute to 'L-sat', ESA's venture in Europe-wide television transmission. France and Germany are bi-laterally developing the equivalent 'TV-sat', leaving Britain, Italy, and five other countries alone in the ESA project.

Italy is also interested in re-organising ESA when the present director Roy Gibson completes his term. "The programme lacks momentum and imagination" said an official. According to Scalia, ESA "should find a strong manager".

In Brussels, Italy would like to link the future of the European nuclear fusion experiment, JET, with that of 'Super Sara' — an experiment on the core 'melt-down' of thermal reactors which would take place at the European Commission's joint research centre at Ispra, north Italy. Britain and other delegations have consistently refused to fund the £35 million experiment, ostensibly because they feel that the costs are too high.

"It would be interesting to know" said Scalia "in what way the countries that oppose such an important programme on reactor safety — one which has been described by America as of great significance — will justify their position to the public . . ."

Germany, an Italian official told *Nature,* had already approached Italy to see if the experiment could be undertaken bi-laterally. Japan has also shown interest. "Whoever can develop a demonstrably safe reactor, will take the world market" said the official. But Italy would prefer that the experiment be undertaken under a pan-European banner.

Criticism of Super Sara has centred on the efficiency of Ispra staff. "We reject that" said the official. Encouraged by the government, Italian unions had sent observers to Ispra, to Brussels, and to Culham where JET is being built, "to assess absenteeism, times of arrival for work and so on". The results put Ispra ahead of Culham, and firmly ahead of Brussels, he said.

"We must have a European programme on nuclear safety" said Scalia. "And at Ispra we have a most important establishment both in the quality of its staff and its equipment." JET and Super Sara, he said, "must either stand or fall together".

Italy appears to be strengthening its stand on European scientific and technological issues. With the change of government four months ago a career diplomat was appointed to the Ministry of Scientific Research as 'chief of cabinet' — the principal advisor to the new minister — and he has been playing an active role in asserting Italy's position in Europe.

The objectives of this exercise may not be entirely a matter of supporting Italy's scientists. The country has taken a strong interest in the industrial aspects of space, having recently announced a £125 million five-year national programme ("fully complementary to ESA's"), and is mindful of the possible applications of the superconducting technology that will be needed in the long term for high energy accelerators. The proof of nuclear safety is related both to sales of reactors and to convincing Italy's public that nuclear power is acceptable.

"Advanced technology" said Scalia "must be a European problem". □

Call for European environment charter

EUROPE'S environment ministers should convene a conference on the environment some time during Italy's forthcoming presidency of the Council of Ministres, Italy announced at a council meeting at Brussels on Monday. The meeting would demonstrate to environmental groups that governments were prepared to tackle environmental problems, said Italy's science minister Vito Scalia last week.

There should also be agreement on a charter announcing the principle of "polluter pays" said the minister, and ultimately, "no pollution".

However, Italy's own record on pollution control is not outstanding. The United Nations Regional Seas Programme, a component of the UN Environmental Programme, recently advised Italy to *lower* its standards for pollution of bathing beaches — because the existing standards were impossibly high. "If Italy applied them, all their beaches would be closed" said a UNEP spokesman. "Better to take a realistic level, and then actually apply it."

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Europe's new space launcher stays on the launch pad

ARIANE, Europe's 200-tonne 3-stage launcher designed to put 1-tonne payloads into geostationary orbit, stayed resolutely on its launch-pad last Saturday during the first attempt to put the vehicle into space. Journalists, watching closed-circuit television screens at the European Space Agency's headquarters in Paris gripped their notebooks excitedly, and radio reporters cleared lines to their news bureaus, as the seconds ticked away to zero. With a few seconds to go, the two "cryogenic arms" which had fuelled the rocket in the previous hours and minutes swung away; there was a cloud of vapur — was it taking off?

No. Ignition was due at zero seconds. Lift-off at +3s. Four seconds, five seconds after zero — was it going to explode, like the first V2s, or like the previous European rocket, Europa, which blew up shortly after take-off? "Mondieu" said the radio man next to me into his telephone. I peered more closely at the screen, thinking he'd spotted something. But then the vapour drifted away, Ariane looked just as before. Anticlimax. ESA officials, long-faced, made for the bar, where the whisky bottles were quickly emptied.

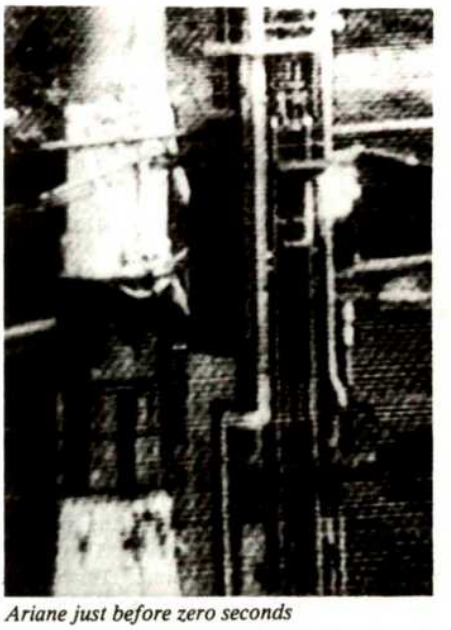
Then the details began to come through. One of the four main engines of the first stage had failed to reach the planned pressure of 9 atmospheres at its combustion chamber — it had reached only 5 atmos — and the computer automatically controlling the last five minutes of launch wisely decided to close the fuel valves. According to some accounts this decision had been made within 0.1s of ignition, accounting for the puff of vapour.

When the disappointment had abated a little, Brian Stockwell, deputy head of the Ariane programme in ESA, said that he'd give 50-50 that it was an instrument rather than an engine failure. "The engines have been fully qualified" he said. Engines early in the development phase had failed to reach design pressure, or had done so more slowly than others, but the problems had been ironed out. The particular engines on this vehicle had never been fired, but they came from a series which was fully proven.

By Monday, detailed records made at the Kourou, French Guiana launch site of the operation of the engines were supporting Stockwell's opinion. An ESA statement said that a malfunction of two pressure sensors on one of the engines "is very clearly shown by the recordings and is attributable to a local overpressure occurring in their feed line shortly after engine ignition, thus putting them out of order. Appropriate steps to avoid a recurrence of such an incident are under study".

Re-launch is planned for next week, the statement says, the time being taken up by re-equipping the vehicle with seals that have been broken and reversing other 'one-way' changes that are made in the last few minutes of the launch sequence. Ariane must also be checked to see if its brief ignition caused any damage.

Officials are therefore hopeful that Ariane will fly with the first and second stages functioning normally. But the third stage, using liquid hydrogen and oxygen, is more doubtful.



Ariane just before zero seconds

While versions of the first and second stages have both been fired successfully in ground tests, the third stage remains to be 'qualified'. In its most successful ground firing, the engines burned only for 90s, instead of the designed 150s. Full qualification is not expected until February, before the launch of a second Ariane; so ESA officials are looking on this first launch as a development flight rather than a test launch. And if of the first four launches planned for the next year, two Arianes successfully put their test packages into orbit, ESA will consider they have a success.

Robert Walgate

UK genetic engineering regulations relax gradually

THE outcome of the UK Genetic Manipulation Advisory Group's programme to assess the risk from recombinant DNA experiments has been a gradual lowering of the categories of physical containment under which many experiments are now being done, according to Sir William Henderson, chairman of the group. The programme, suggested by Dr Sydney Brenner of the MRC Laboratory of Molecular Biology, Cambridge at the end of 1978 (*Nature* **276**, 104; 1978), identified the type of host vector system as a crucial factor in designing safe experiments.

The development of safe host vector systems over the past year has meant that about 90% of recombinant DNA experiments can now be done under the first two categories of physical containment laid down by the Williams guidelines. Experiments which still come under categories III and IV are mainly those involving the expression of toxins or hormones.

GMAG had been slightly embarrassed, said Sir William when introducing the group's second annual report last week, when the National Institutes of Health in the US proposed 'relaxing' its guidelines about a year ago. At that time, genetic engineering experiments in the UK still came under the more stringent guidelines laid out in the Williams report. Now, however, it was extremely difficult to detect the practical difference between the two systems, said Sir William. The percentage of experiments coming under categories I and II had increased steadily over the past few months from 66% in July to 90% in December.

The procedure of notification of experiments has also changed over the past year. Notification to GMAG and the Health and Safety Executive is now compulsory. Synopses of experiments together with details of the DNA and host vector systems to be used have to be submitted to both bodies and local genetic manipulation safety committees have to recommend the category under which they think the work should be done. Work under categories I and II can begin as soon as GMAG and HSE are notified.

Initial concern that local committees would be incapable of judging categories has not been substantiated. In only one case, said Sir William, did GMAG have to increase the category of containment and on a few occasions it had even recommended lower containment with the used of improved host vector systems.

Despite the success of the past year, however, the members of GMAG still see a need for careful monitoring of recombinant DNA experiments. Most experiments are now done with disabled host vector systems which would not have been developed if GMAG had not existed, said Professor Bob Williamson of St Mary's Hospital Medical School, London and a member of the group. If GMAG ceased to exist now most of the awareness amongst scientists of the need for safety in experiments with recombinant DNA, which it had helped to create, would vanish.

The major problems which GMAG will be tackling over the coming year include industrial scale-up — one case has already arisen — and genetic manipulation in crops.

Judy Redfearn

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Poland steps up research into mining safety

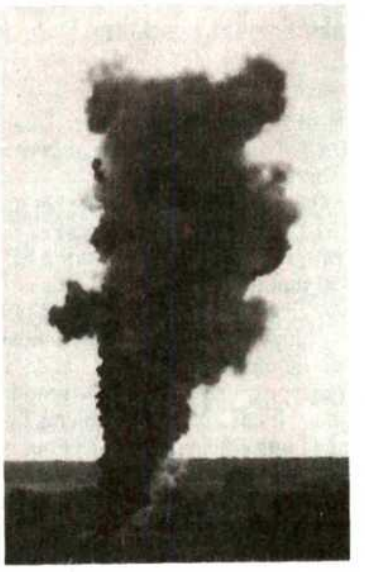
POLAND celebrated St Barbara's day, (4 December) — the traditional miners' festival — in the shadow of three major disasters that cost the lives of 66 Silesian miners in October. Not surprisingly, the official celebrations stressed the importance of mining safety. Speaking at Katowice, the mining centre of Silesia, party leader Edward Gierek, himself a former miner, called upon "all Polish scientists . . . to develop research into greater safety in mining". At the same meeting the Minister of Mining, Wlodzimierz Lejczak, announced that this year Poland is spending some 15,300 million zloty on mining safety.

Coal accounts for 95% of Poland's energy needs and is a major hard-currency earning export, bringing in some $1,700 million annually; total production now runs at some 200 million tonnes annually. However, Poland's mines are particularly hazardous. Recently, Dr Jerzy Matuszewski of the Central Mining Institute in Katowice told *Nature* that in virtually all Polish mines there is a considerable methane hazard. Moreover underground mechanisation is increasing the dust hazard, and the great depth of the pits — about 1000m — exacerbates the risk of rock-falls.

Dr Matuszewski is director of the institute's own experimental mine — appropriately named "Barbara" — where a network of galleries (5 km in all), provided a test-site for the study of gas explosions.

Researchers also study dust control at the "Barbara" site. The dust control section produces spectacular demonstrations of the almost instantaneous combustion of methane when mechanical cutters are used without the water spraying now obligatory in mines using such cutters. Less spectacular, but equally important, are the routine tests on the combustability of conveyer belts, the monitoring of surface movements, the design of protective clothing, and various anti-silicosis measures.

Mining safety research, in fact, extends far beyong those institutes specifically associated with mining. The Institute of Geophysics of the Polish Academy of Sciences, for example, is currently carrying out a geodynamic survey to attempt to predict earthquakes in mining areas. A special fire-extinguisher recently introduced in hard-coal mines was developed in collaboration with the air-force technical institute. And the Department of Electromagnetic Wave Theory of the Technological Institute of the Academy of Sciences have produced a system of radiocommunication for mines.



Experimental explosion: Poland stresses mining research after major pit disasters

So much for the physical hazards. What about psychological stress? Although there are some psychological experiments going forward — mostly psychomotor studies — little is said officially about the role of physchological stress in mining accidents. And no official statistics of day-to-day accidents are published — although 'unofficial' estimates place the 1978 fatality figure at around 180. One mining official suggested that "production targets" were the greatest single cause of accidents — "but there is little can be done about that!"

Mr Gierek, in his own tribute to the miners' festival, spoke of the new "four-shift" system (three shifts a day, plus one shift entirely off). This was officially introduced two years ago, is being implemented in an increasing number of mines, and is aimed at "improving our miners' working and living conditions". Mr Gierek failed to mention that all three of the mines involved in the October disasters had "four-shift" schedules.

Computers can replace maths for scientists, says Soviet professor

COMPUTERS must inevitably change the whole structure of mathematical training, from scientists down to schoolchildren, according to Professor Aleksandr Kitaigorodskii. Writing in the prestigious Soviet weekly, *Literaturnaya Gazeta* (No. 43, 1979), he envisages a future with a small computer on every classroom desk, where pure mathematics will become a form of art and "culture", and problem solving will have virtually vanished from the academic syllabus. With the advent of the computer as "co-respondent", says Kitaigorodskii, the long "marriage" of mathematics and science is ripe for "divorce".

His article is clearly intended as something more than idle futurology. A well presented "press campaign" is a standard Soviet gambit for informally airing a proposed major policy change. A few issues later, (no. 49), an article by Professor M Evgrafov of the Mathematical Institute of the Academy of Sciences of the USSR ostensibly presented a counter-argument. This, however, while counselling caution in planning reforms, only served to reinforce the theme of mathematical irrelevance. Evgrafov claimed in particular that "not a single result obtained in pure mathematics" has had any practical applications for at least 50 years, while by that time "90%" of all such results have now been completely forgotten; and even at the time of its discovery, the "most elegant mathematical result" will be understood by "not more than 100 persons". Since, moreover, so few people are capable of "creative mathematical work", he finds the current insistence on mathematical training in higher education and research instutites, "wanton", "rigid", and "useless".

As for problem-solving, he suggests that in the future this will become pre-eminently the domain of the programmer ("a person of middle-level qualifications") and only if the problem has been wrongly formulated will it be necessary to revert to the "tried



An apple for the programmer

and tested" method of the "Old Testament" — calling in a highly qualified mathematician.

More vehement counter-agruments have been reduced so far to small type at the foot of a column. They deal mainly with a computer's inability to "solve" anything, or, more specifically, to deal with continuous or infinitesimal processes.

A somewhat more original suggestion is advanced by A Badaev, a Candidate of Technical Sciences (roughly equivalent to a PhD) from Penza. Without the common language of mathematics, he says, science would soon be reduced to a state resembling "the well known Biblical legend of the Tower of Babel".

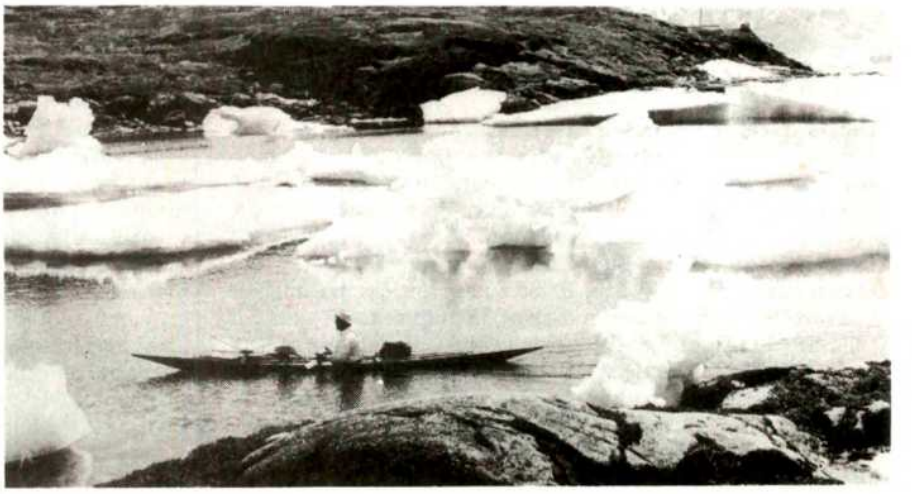
Vera Rich

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Native peoples oppose uranium mining

Representatives of native peoples met in Denmark recently to discuss the problem of uranium mines on their lands. **Roger Moody** reports



John F. Racmussen.

The Narssarsauq seaboard: Greenland has vetoed further development of uranium

THE First International Conference on Uranium Mining in the Third and Fourth Worlds was remarkable, not only in its attendance of 500 anti-nuclear activists and representatives from the developing world, but in the fact that it occurred at all. Held in Copenhagen on 25–27 October, the conference was organised at short notice by three Danish anti-nuclear groups.

Taking advantage of the fact that two US Indian delegates, Winona LaDuke and Herb Blatchford were visiting Europe (see *Nature*, 11 October 1979), the Danish anti-nuclear group 'OOA' issued invitations to activists and representatives of native peoples from Africa, Australia and Greenland.

Just three weeks before the conference, the ruling Siumut party in Greenland had strongly condemned the involvement of their economy in the nuclear age precipitated by an energy-hungry EEC. In particular Siumut vetoed further immediate development of the Narssarsuaq uranium deposit (27,000 tonnes) on the far south-western tip of Greenland. And by coincidence, two Australian Aborigines also happened to be visiting Europe at the time of the conference. They were trying to set up an Aboriginal information centre one of whose aims would be to work for a moratorium on the development of the Ranger, Nabarlek and Jabiluka deposits, all on Aboriginal land in the deep Australian north.

Mick Miller of the North Queensland Land Council, the largest single body representing any of Australia's original people, talked about the struggle for land rights, pointing out that Aborigines had been aware of the potentially devastating nature of uranium "thousands of years ago". Using the powerful images of Aborigine culture, Miller told of an Aborigine belief about the land on which the Ranger mine is due to go on stream around 1981. The story has been told through generations that if the earth is disturbed on this spot, *gabo djang* (green ants) will rise through the crust and destroy the world.

Herb Blatchford's forebears had also sensed the enormous importance of the 'Four Corners' area into which they moved and settled 4000 years ago (the Four Corners comprises New Mexico, Colarado, Utah and Arizona). However, when uranium mining started in New Mexico (initially for US nuclear weapons) about 30 years ago none of the Navajo or Pueblo people in the Four Corners was told the full extent of what was going on: "We asked — why do we feel the nausea, see our hair fall out, why do our teeth weaken, why do we get up in the morning and have no breath? We went to books, looked back at history. Then we went to the Japanese. We went to Enuwetok atoll and asked them. Then we went to the Nevada Platte people. They all gave us the same answer: *Radiation*."

Greenland's delegates seemed unaware of the full implications of allowing uranium mining on their land. Robert Petersen (surely the only Inuit eskimologist in the country) received polite applause when he reflected the Siumut position that mining should only proceed "if all the dangers of radioactive pollution and waste dumping are solved". Of one thing, Petersen was quite certain: "The mine cannot be a solution to the increasing unemployment among our people. If Narssarsuaq goes ahead, it will produce only a few jobs for our people, and bring in even more outsiders."

Another Greenlander took issue with Petersen on this, and became the only delegate to approve of mining "provided the safety problems are solved" since "we must have an economic base on which Home Rule can work". Jorgen Hertling is a member of the Atassut party which has strong links with Denmark, Greenland's former colonial rulers.

The two high spots of the conference were both unexpected. Immediately after Hertling left the platform — to stony silence — two young Greenlanders rose to announce (in both Inuit and English) that they had just decided to form an anti-uranium mining group in their country. During the next few months, they will travel round the scattered seaboard communities mobilising against the Narssarsauq project, and strengthening the veto already imposed on it. (There is a widespread fear among Greenlanders that the Danish government will override the veto, under pressure from the EEC.)

But most surprising was the address given by the only 'Third World' delegate, Hadino Hishongwa from Namibia. Hishongwa is the South West Africa Peoples' Organisation (SWAPO) representative in the Nordic countries, Austria and West Germany. Although opposition to foreign control of Rössing, the world's largest operating uranium mine (1978 production: 5000 tonnes uranium oxide), received majority support in the UN Assembly four years ago, SWAPO has never argued against uranium mining as such until now.

"Uranium is an evil", announced Hishongwa. "It is a threat to Namibia and a threat to the entire world. SWAPO doesn't want to exploit it, even when we are independent."

As well as the Rössing mine (in which Britain's Rio Tinto Zinc Corporation has a 46.5% beneficial interest), South Africa's huge General Mining Corporation and France's Aquitaine are involved in prospecting further uranium lodes in the Swakopmund area, and Namibia's actual deposits could rival those in Gabon and Niger.

So ended a conference which was rather short on facts, somewhat higher on energy and certainly bounding with rhetoric. Whether native or 'natural' peoples in the developing world live on 80% (as was claimed by the conference organisers, OOA) or 40% (my own estimate) of the West's reasonably assured uranium reserves, they are certainly the most vulnerable groups. And if uranium exploration and mining expands by up to 150% — as this year's Uranium Institute's annual symposium proposed it should — future conferences should see greater representation from native peoples who did not hear about, or could not get to, the Copenhagen gathering. □

Roger Moody is an editor of Natural Peoples News *published by Colonialism and Indigenous Minorities Research/Action*

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news in brief

Dioxin traces found in soldiers exposed to defoliant: Research workers at the Veteran's Administration in Washington announced last week that tests had revealed the presence of small amounts of dioxin in fat tissues taken from men who were exposed to the defoliant 2,4,5-T during the Vietnam war. Dr Lyndon E Lee told a public meeting of the VA's Advisory Committee on Health-related Effects of Herbicides that analysis had revealed traces of dioxin ranging from three to 57 parts per trillion in fat tissue taken from 33 individuals, including a control group of 10 who had not served in Vietnam. However he added that the amounts of dioxin, a highly toxic contaminant, were so small that it was impossible to attach any significance to them.

Meanwhile President Carter has announced the setting up of an interagency working group to study the long-term health effects of exposure to Agent Orange and other herbicides which were used extensively during the Vietnam war, and which many veterans are now claiming responsible for complaints ranging from liver disorders to chromosomal damage. According to the White House the working group will have the major responsibility for making public the results and implications of research on the health effects of phenoxy herbicides and their contaminants.

London School of Hygiene and Tropical Medicine fears closure: London University's School of Hygiene and Tropical Medicine may close if the government's proposed increase in fees to foreign students goes ahead. The school now charges overseas students £1200 per year which would have to increase to £5000 next year and to £7000 by 1982-1983 if the government goes ahead with its plan. Of the school's 249 full time students and 1023 part time students 58% are from overseas from a total of 85 countries. The school is a postgraduate institution offering MPhil, MSc and PhD degrees and is knwon for research and teaching in tropical diseases as well as community medicine, occupational medicine and epidemiology and statistics. Gordon Smith, dean of the school, said that the probable loss of overseas students was not just a question of money. "Our students are a resource of considerable magnitude. They are experienced and knowledgable and the school's research programmes could not function without them." Smith also stressed that since the Soviet Union was the only other country offering research and training in tropical medicine at a "reasonable fee" that a policy directed against overseas students might have "undesirable foreign policy implications".

Other internationally known schools of London University threatened by closure because of the proposed increases include the School of Oriental and African Studies with 35% overseas students and the London School of Economics with 37% overseas students.

ASTMS calls for MRC tenure reforms: The Association of Scientific, Technical and Managerial Staffs is discussing proposals for extensive reforms in the UK Medical Research Council's tenure procedures. A working paper circulating to ASTMS/MRC national advisory committee affirms ASTMS general policy that "potential dismissal, including the end of fixed term contracts, should be the subject of negotiation and not be solely at the employer's discretion". The proposals call for changes in both new appointments and tenure procedures. Present non-renewable three year appointments would be replaced by three year fellowships with a one year renewable option, a retirement contribution, no age limit, no waiver clause and would end with a "substantial cash gratuity if the fellow is not taken on to permanent staff". The proposed tenure procedures for scientific staff would replace the present five year scheme with a one year probationary period followed by a tenure decision. The tenure decision would be made with the candidate having full access to submitted reports and in the case of denial reasons would have to be given.

UK National Physical Laboratory Chemical Division to close: A 4% cut in the budget of the National Physical Laboratory is to be applied solely to the division of Chemical Standards. Chemists in the division were told that a decision had been "made centrally" to close two sections in the division because "the jam had become too thin to spread further". The two sections, Reference Materials and Thermodynamic and Phase Equilibria Data, employ two thirds of the Division's 60 scientists and the entire division will close as a result. The scientific staff has been given two years and three months to find other jobs. It is not clear whether the NPL will employ them since the Chemical Standards Division is the only one devoted to chemistry at the NPL.

UK energy report calls for development of fast breeders: A major Department of Energy report on energy technologies for the UK regards the fast breeder reactor as the only economic solution to Britain's electricity needs over the next fifty years. On the basis of the assumptions made in the report it is estimated that it will ultimately be economic to install nuclear power to generate between 80% and 90% of public electricity supply. The fast breeder is seen to be the likely solution for the ensuing world-wide competition for uranium. The report says "Only by introducing the fast reactor on a large scale by the early decades of the next century can the world demand for uranium be brought within credible bounds". Fusion power is seen to be unrealistic and alternative sources of energy are regarded only as insurance against shortages. *Energy Technology for the UK* Vol 1 (£3.00) Vol 2 (£5.75). HMSO.

UK to provide free science consultancies for industry: A scheme to facilitate "industrial exploitation of innovations originating in research" has been approved by the Science Research Council and the Royal Society. The two bodies will provide £100,000 a year for ten fellowships to enable academic scientists to "work directly in the productive process" and for industrial scientists to learn new basic research techniques. The scheme is expected to be effective in "growth technology areas" in which there is "quite a distinct demand on the industrial side". Although the scheme is intended to be "flexible" genetic manipulation techniques are expected to be in high demand in industry circles.

BSSRS calls for new safety procedures for hospital radiation hazards: The British Society for Social Responsibility in Science Hospital Hazards Group has issued a report calling for new radiation limits and redundancy agreements for hospital workers exposed to radiation. The report suggests that trade unions should negotiate local limits at each hospital that are substantially lower than established upper limits since "all research indicates that the majority of exposure can easily be kept at 1/10 of the current limits".

The report also calls for trade unions to negotiate lay-off agreements whereby workers who receive their maximum allowable yearly dose of 5 rem are transferred to new jobs without loss of pay or seniority. In addition the report recommends that protective safety clothing be a measure of "last resort". Substitution (e.g. ultra sound for x-rays) and containment or shielding are seen to be superior safety measures. *Radiation Hazards in Hospitals.* British Society for Social Responsibility in Science, 9 Poland St, London W1. 18p.

Correction: In the correspondence "Fast reactors will be problematical and expensive" (22 November, page 358) there was an error in the fourth sentence of the second column. It should have read '. . . spokesmen for the Nuclear Installations Inspectorate stated "we have indicated the seriousness with which the NII views the possibility of whole-core accidents in fast reactors. The consequences are potentially worse than for major accidents in a thermal reactor system . . .".

Recombinant DNA: have recent experiments assessed all the risks?

Experiments to test the safety of cloning viral genomes in *E. coli*, published earlier this year, have allayed fears about the risks of recombinant DNA. But, argue **Barbara Rosenberg** and **Lee Simon**, the experiments actually indicated some worrying aspects of the behaviour of recombinant DNA.

TWO reports on the assessment of risk from recombinant DNA were published in March, 1979 in *Science* (**203**, 883; and 887). These experiments were designed "to evaluate certain postulated machanisms of possible biohazard stemming from recombinant DNA research with *E. coli*". The authors "view these results as being highly reassuring with respect to the safety of cloning viral genomes in *E. coli*". This statement, in our opinion, reaches far beyond the results presented. The new data actually confirm several aspects of hazard mechanisms that have been postulated, but they do not address other aspects that must be investigated before the overall hazard can be evaluated. Nevertheless, the published conclusion has been widely accepted and the reports have been cited as cause for the relaxation of fears about risk to the public of gene-splicing experiments (for example *The New York Times* 2 May; *Nature* 15 February, page 505).

A team headed by Wallace Rowe and Malcolm Martin of the National Institute of Allergy and Infectious Diseases did the research at Fort Detrick. Their experiments tested the ability of certain recombinant molecules containing polyoma virus DNA to cause viral infection in mice (polyoma virus grows readily in mice and is easily detected). Subsequently, a more cautious report of very similar experiments, sponsored by the European Molecular Biology Organisation (EMBO) appeared in *Nature* (28 June, page 811). The EMBO group prepared the same types of recombinants and tested for virus production in cultured mouse cells; they plan to test later for infectivity in animals. The assay used in both studies detected infectious virus particles produced by the recombinant molecules, and so required total functioning of the recombinant polyoma virus DNA to produce a positive result.

Only a relatively uncommon type of recombinant molecule was found to produce infectious particles. (This contained a tandem polyoma insert, which because of its head-to-tail double structure is capable of regenerating free circular polyoma DNA within the cell.) Ordinary recombinant molecules with a single polyoma insert did not do so. This is indeed expected in the light of current molecular biological knowledge. To produce infectious virus from a single polyoma insert would require specific mechanisms for excision or replication which are not known to exist.

Furthermore the particular recombinants chosen for study are incapable of the full genetic function necessary for virus production. They were all constructed with enzymes that cleave polyoma DNA within a transcriptional region and in the resulting recombinants a part of the genetic information is disjoined from its control region and could only be reconstructed by regenerating free circular polyoma DNA. The production of free virus particles by recombinants with a single polyoma insert would thus require a highly unlikely intracellular event. The observed lack of infectivity conveys a false sense of security which arises from the method chosen to evaluate the possible biohazard.

In fact the production of complete, infectious DNA viruses by recombinant DNA, as tested in these experiments is one of the least probable hazards of recombinant DNA technology. There are other dangers in cloning viral DNA that are at least as serious, and far more probable: in particular the possibility that one or more viral genes could function without the production of a complete virus. In the case of tumour viruses such as polyoma, partial genetic functioning of the virus DNA is considerably more dangerous than the production of infectious virus. The action of a single viral gene can transform a cell towards malignancy and consequent tumour formation, whereas complete virus function leading to a productive infection is harmless to mice.

The functioning of single genes from other sources, for example toxin-producing genes, could also be hazardous. This is the kind of risk posed, in general, by the cloning of eukaryotic DNA (DNA from organisms other than bacteria). Single gene function is a 'worst case' hypothesis — it suffices, for example, for a scenario which envisages the spread of microbial-borne cancer. A test for single gene function in recombinant molecules therefore provides a much better basis than infectivity for conclusions on the safety of the technology.

The team led by Rowe and Martin have now published information on this subject (*Science* **205**, 1140; 1979). Their results show that polyoma virus DNA in recombinant molecules induce tumours in newborn hamsters with a frequency similar to that of non-recombined polyoma DNA; the recombinant molecules can therefore function genetically in mammalian cells. These positive results indicate that recombinant DNA can be highly hazardous if it is brought into appropriate contact with an animal.

In these tumour induction experiments, 19% of animals injected with intact, uncleaved polyoma DNA developed tumours, and 19% of animals injected with intact tandem-insert polyoma DNA also developed tumours; intact recombinant λ or plasmid DNAs with single polyoma inserts gave variously 0.6 and 9% tumorigenicity (Israel *et al. Science* **205**, 1140; 1979: Table 2). The overall variation in the tumorigenicity of intact recombinant molecules, 0-19%, is a result of the small numbers of animals seen; similar variability was seen in similar conditions for tumorigenesis by free intact polyoma DNA (*J. Virol.* **29**, 990; 1979; Table 5).

An accurate, quantitative determination of tumorigenicity is not possible from the data given, but it is clear that for both single and tandem-insert recombinants the tumorigenicity is of the same order of magnitude as that of free polyoma DNA. One can conclude that the insertion of polyoma viral DNA into a vector molecule from a different source does not significantly change its tumorigenicity and is, therefore, not a safeguard. Moreover, recombinant DNA can clearly enter hamster cells and the region of the inserted DNA required for tumour induction evidently finds genetic expression. This indicates the potential genetic activity of recombinant molecules containing DNA from any source, not just viruses.

The successful infection experiments with recombinants containing tandem polyoma inserts also indicate that recombinant DNA can enter mouse cells. The authors point out that the recombinant DNA is not recognised there as foreign and that the polyoma DNA retains its biological activity after cloning in bacteria. These experiments also reveal a new element in the hazard that may be posed by genetically active recombinant molecules. The recombinant molecules became 100-1000 times more infective when they were enclosed in λ-phage particles (*Science* **203**, 887; Tables 2 and 3), although it is not emphasised in the text. The phage coat apparently protects the DNA from degradation and facilitates its entry into mouse cells. Thus, polyoma-λ recombinants, both single and tandem are endowed with a large advantage over free polyoma DNA. A close comparison of the polyoma dosages in free DNA and λ-phage particles in the tumour-induction experiments (*Science* **205**, 1140; Table 2)

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confirms this advantage. Thus, if *Escherichia coli* containing polyoma-λ recombinants should come into contact with appropriate animal cells, λ particles possessing recombinant DNA molecules could readily enter the cells and act genetically to induce tumours.

The key question now is whether *E. coli* (or other hosts) containing recombinant DNA can provide a new route by which viral or other genes could find their way to sensitive species.

This question is not addressed in those risk-assessment experiments undertaken so far, although it must be answered before we can be reassured as to the safety of cloning recombinant viral DNA in *E. coli*. Although one of the papers from Rowe and Martin's laboratory states that *E. coli* containing polyoma-recombinants were not infective when injected into mice, this is misleading because they tested too small a dose of the recombinant contained in *E. coli*. In the case of free recombinant DNA a larger dose is required (their Table 2 and 3). (The same is true in the tumour-induction experiments.)

E. coli containing polyoma recombinant DNA induced no tumours (*Science* **205**, 1140; 1979; Table 1) but for technical reasons the polyoma dose contained there in was an order of magnitude lower than the minimum dose required for measurable results with the number of animals used, as shown by the DNA data (Table 2). Moreover the authors point out that experiments involving injection of live *E. coli* may tend to be biased in favour of negative results.

Since most of the animal data were obtained by injection, the relative infectivities or tumorigenicities obtained in this way reflect the ability of the DNA molecules (either free or in phage particles or bacteria), to enter and act in mouse cells with which they have artificially been placed in contact. But injection is not a normal mode of accidental exposure of an animal to bacteria.

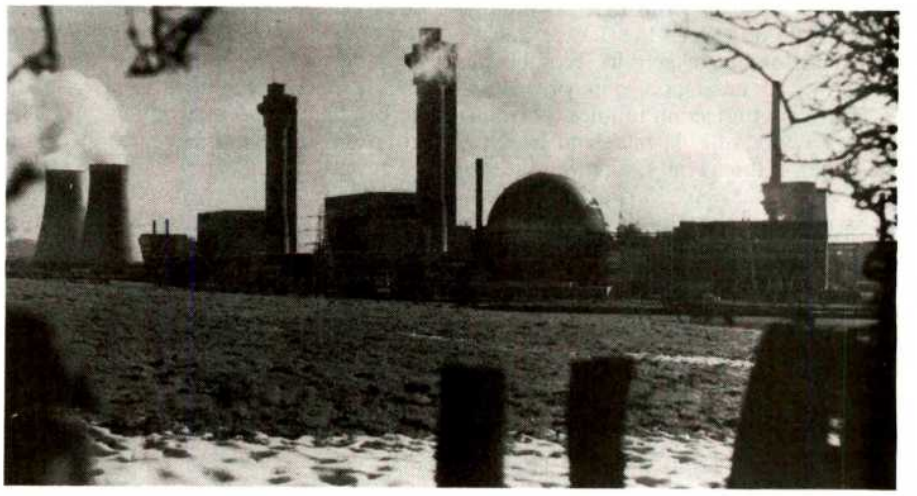
There are many normal ways in which *E. coli* can accidentally enter mammals and proliferate at various sites; urinary tract infection or gut colonisation are typical examples. If an *E. coli* strain containing a recombinant were able to colonise an animal in these or other ways, or transfer the recombinant to another bacterium that could do so, the amount of the recombinant could eventually be greatly amplified internally. The animal would then be exposed to the recombinant continuously over a long period of time, thereby increasing the risk substantially, even if the event is of low probability when infection by polyoma virus itself is considered. Via the recombinant, polyoma genes would be given a new route of access to the animal.

E. coli host-vector systems have been developed with the aim of minimising this possibility, but their reliability has not yet been demonstrated. Indeed, risk-assessment experiments in progress under contract to the US National Institutes of Health indicate that survival of EK2 strains in the human gut (other sites have not been systematically tested) and in the environment is considerably greater than originally anticipated, and plasmid vectors can increase survival. The likelihood that recombinant EK2 strains may be able to transfer genetic information to indigenous microorganisms in various natural environments may therefore be significant, but this has not yet been adequately tested.

The extent of the hazard posed by a potentially functional recombinant clearly depends on whether the host-vector system used can provide a new route to the animal, or plant, or to man. The main import of the present risk-assesment experiments which in effect rule out any automatic safeguards at the levels of cellular entry and gene function, is to give new importance to the question of new routes of genetic access that could be provided by *E. coli* or other cloning hosts. Until this has been fully studied, there is no basis for reassurance on the risks of recombinant DNA. □

Nuclear power: the threat to personal freedom

Robin Grove White looks at the implications for civil liberties in the UK of a major increase in nuclear power.



The UK government's proposal to expand the nation's nuclear capacity will increase debate on the long term impact of nuclear power on civil liberties. This issue has already become central to the debate in other European countries.

The Royal Commission on Environmental Pollution first raised the issue of nuclear power and civil liberties in its sixth report (Cmnd 6618) in 1976. Together with subsequent independent studies, the report argued that a major commitment to nuclear power, and in particular to the plutonium fuel cycle, would require extensive security measures to safeguard nuclear materials such as plutonium from theft and sabotage. These safeguards would damage relations between the individual and the state.

Extensive vetting of workers inside and outside the electricity industry, the growth of unconventional armed protection for nuclear facilities and materials, wide-ranging surveillance of dissident groups and constraints on the flow of information about nuclear power to the news media would all contribute to increasing authoritarianism.

The government and the nuclear industry were not impressed with these arguments. In June 1977, the Department of Energy said that future nuclear plant could be designed to eliminate many of the potential civil liberties' hazards. Industry spokesmen such as Sir John Hill Chairman of the Atomic Energy Authority (UKAEA) went further: a combination of technical fixes such as radioactive 'spiking' of fuel elements, siting fuel fabrication plants and power stations in the same place and physical obstacles at vulnerable points in the fuel cycle would obviate the need to intrude on civil liberties. In any event, it was maintained, thieves were unlikely to be interested in materials such as plutonium.

Such confidence is increasingly hard to endorse. No such technical fix has been found to be politically or economically feasible and there have been ominous developments.

On 26 October 1979, the first International Convention on the Physical Protection of Nuclear Materials (for peaceful purposes) was concluded in

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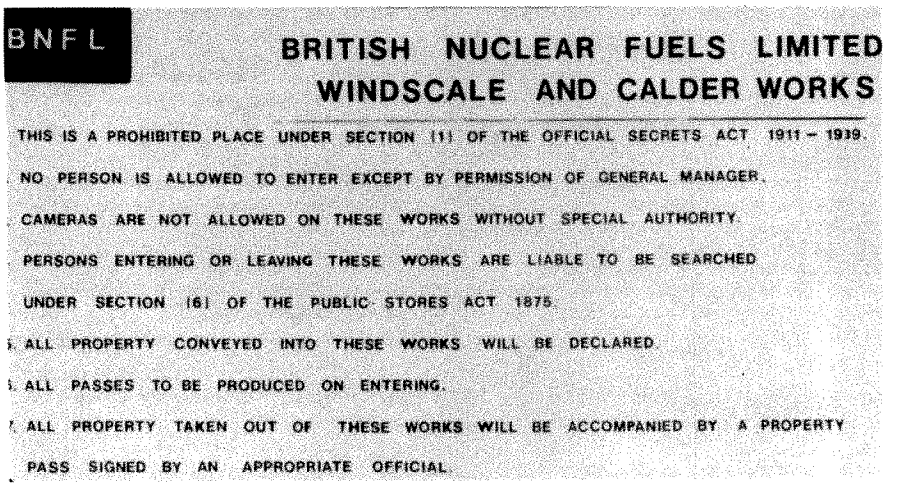
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Vienna. The convention established draconian measures to protect the use, transport and storage of civil nuclear materials, including the exchange of information between national security authorities, rigorous vetting of individuals with access to sensitive points in the fuel cycle, arming of guards and an agreement that all exchanges of information concerning nuclear security should be confidential. The convention is far franker in its recognition of the hazards of civil nuclear power than the UK.

The security community has recently said that surveillance, vetting and secrecy will play a growing role in economies opting for nuclear power. The 1978 Rand Corporation Report, *Attributes of Potential Criminal Adversaries of US Nuclear Programs*, stressed the limitations of physical security in guarding against theft or sabotage of civil nuclear materials. Rand lays emphasis instead on the need for more stringent security clearance procedures, for secret armed police and for a pervasive air of 'uncertainty' (i.e. secrecy) to thwart nuclear hijacks.

This agrees with a senior police official who recently said at a high-level UK seminar, that, for nuclear materials, investment in intelligence tends to be more cost-effective than an equivalent investment in physical containment.

An indicator of the growing gap between the UK's earlier official reassurance and



hard reality is the growth since 1976 of the UKAEA's Special Constabulary. This private armed police force operates on a constitutionally unique basis, its actions shrouded from public (and parliamentary) view for security reasons. Justice, an all-party association of UK lawyers, has commented that the constabulary's structure "conflicts with all our traditions of civilian and politically accountable policing".

In February 1977, Friends of the Earth and others asked the then Energy Secretary, Tony Benn, to comment on the likely scale of the constabulary's expansion to meet the security demands of a hypothetical 127GW nuclear programme. His department's considered reply was that "not more than a few hundred" extra armed constables would be required to guard even this massive programme. However, between 1976 and 1979, the constabulary's strength increased 50%, from 400 to 600 — and at a time when there have been no additions to the civil nuclear programme.

There have also been developments in the levels of vetting now thought appropriate for staff of relatively low skill in the nuclear fuel industry. There has always been extensive vetting of the personal associations and political affiliations of employees of the UKAEA and British Nuclear Fuels Ltd. The extent of such security screening has now become a significant irritant in union-management relations. At a conference on Trade Unions and Nuclear Power in November 1978, a senior official of the Electrical, Electronic, Telecommunications and Plumbing Union (himself no critic of nuclear expansion) expressed dismay at the escalation of security vetting at Windscale.

Recent foreign experience suggests that intensified surveillance of bodies opposed to nuclear power is a likely concomitant of nuclear expansion. Reports from West Germany, Australia and the United States point to cases of spying and infiltration of campaigning groups. Over the past few months in the UK, commentators as various as the *Daily Telegraph*, Sir Fred Hoyle and the Institute for the Study of Conflict have pointed to the potential advantages to the Eastern Bloc of a strong UK anti-nuclear movement. It is likely that such views are already reflected in UK security, given the problems that could arise from disruption of the nation's energy supply and the dangers of nuclear materials in the wrong hands.

But how far will such surveillance extend? And how is it to be kept within bounds? Two rival imperatives clash. Public understanding of the full implications of nuclear power demands openness about civil liberties' before irrevocable decisions are taken. But the imperative of security (itself the very source of the difficulty) dictates silence.

Weighing evidence about this conflict in his 1978 report on the Windscale inquiry, Mr Justice Parker could offer "no solution at all". He opted for security and silence.

In June 1977, the Energy Secretary, Tony Benn, stressed that "the government are well aware of the need for discussion of such an important issue to take place on an informed basis . . . [They] will make every effort to ensure that adequate information is available. They are confident that it will be possible for Parliament to perform its traditional function in this area". Yet just six months later, in reply to a Commons question about the range of security measures needed for fast breeder reactors, Mr Benn was brutally laconic: "I can only give the classic answer: that I am advised that the increase in surveillance and vetting will not go above a normal level".

Powerful arguments for greater openness have been made. In a recent article in *International Relations*, Cambridge historian Christopher Andrew urges: "As the dreadful day of the terrorist with a nuclear device in his suitcase draws gradually and inevitably closer, intelligence surveillance of potential terrorist groups will have to be increased. And it is essential that increased surveillance should have the support of informed public opinion . . . The only alternative to a public informed about the intelligence services is a public misinformed about and suspicious of them".

But is such openness credible, or even desirable? Each additional nuclear commitment compounds the tensions over civil liberties as it leads towards ultimate reliance on the plutonium-fueled fast breeder reactor. In this way, argue the civil libertarians, new thermal nuclear power stations are contributing towards new and unhappier relations between the individual and the state. In April 1977, for example, the International Commission of Jurists, a group of leading lawyers concerned with human rights and the law, met in Vienna and expressed "grave concern about the dangers to human rights created by nuclear weapons and materials and nuclear power programmes". It called on all governments to "take these dangers into account before forming a policy on nuclear power".

Tony Benn was undoubtedly worried about these issues when he was Energy Secretary, as some of his public statements testify. But there is little in the public record to suggest that civil liberties have weighed significantly in the decisions of David Howell, his successor.

It is conceivable that the pursuit of nuclear power will damage civil liberties less than would the pursuit of an alternative long term energy strategy, such as the low energy growth programme outlined earlier this year by the International Institute for Environment and Development. But the government has certainly not demonstrated this.

The most important social dimension of nuclear technology may be going by default in Whitehall. □

correspondence

Stench on the New Jersey Turnpike

SIR, — The central point in Thomas Jukes' recent review of *Malignant Neglect* (8 November, page 166) — that the book treats facts cavalierly — is amply documented by the quotations he cites. I should like to point out, however, that Professor Jukes' own attitude to facts is not beyond criticism.

Jukes quotes the book's description of the "stench" and emissions from the large petrochemical complex near the New Jersey Turnpike, which (it implies) is related to 14,000 cancer deaths in New Jersey. He then dismisses the statements on the grounds that he once drove the same route at 6.45 pm and observed neither stench nor emissions, apart from steam.

Having myself travelled this route, by car or train, at least 50 times over the past 20 years, I can assure Professor Jukes that the petrochemical stink in the area is no man's invention. Since I did not keep score, I cannot swear to how often I experienced it — but it was often enough for the experience to become an absolutely expectable feature of the trip.

Jukes' own account provides a clear explanation of why he smelt nothing — assuming, of course, that the complex was in operation at 6.45 pm. "To the west," he notes, "there was a brilliantly clear sunset in which a range of hills, 30 miles away, was sharply etched against the sky." That degree of visibility could exist only amid a continental polar air mass entering the area — which is normally associated with northwesterly winds. The complex lies southeast of the highway.

New Jersey does in fact have one of the highest cancer rates in the US. Whether this has any connection with emissions from the chemical manufacturing facilities with which northern New Jersey is sown has yet to be established. However, a number of reputable authorities, including the US Public Health Service, consider the possibility real enough to be worth investigating.

Yours faithfully,

ROBERT CLAIBORNE

New York, US

Research ceased during the Cultural Revolution

SIR, — Having recently returned from working at the Institute of Vertebrate Palaeontology and Palaeoanthropology in Peking I would like to challenge Tilman Spengler's assertion (15 November, page224) that this was one of the institutes of the Chinese Academy of Sciences that "worked very well" during the Cultural Revolution. Certainly the institute was not physically closed down, but a research institute where no research is permitted, can hardly be said to be working well. By 1972 research activity was starting up again, but for six years nothing was heard of vertebrate palaeontologists or their work. The Chinese vertebrate palaeontology journal *Vertebrata Palasiatica* ceased publication in 1966 and did not reappear until 1973. The impression gained from my Chinese colleagues was that the Cultural Revolution was a period of enforced isolation and stagnation.

Finally, I would like to comment upon the statement attributed to the three Chinese scientists visiting Britain: 'In China we tend to work more collectively. It is unheard of for an individual to work alone . . .'. I suspect this comment may well have arisen from a semantic misunderstanding. Certainly from my observations, Chinese scientists in my own field, at least, work exactly as they do in Britain. The individual scientist is employed with others in an institute, will collect raw data jointly with colleagues, will discuss his or her results with colleagues, may cooperate with others to produce joint papers, but in the main does scientific research alone. This much is evident from even a cursory glance at the authorship of Chinese scientific publications.

Yours faithfully,

L.B. HALSTEAD

University of Reading, UK

China has many aspects

SIR, — In your introduction to the two reports on "China: the legacy of the Cultural Revolution" (15 November, page 224), you used the phrase "views of China are as diverse as its visitors", and seemed to imply a disagreement between myself and Tilman Spengler. I would like to point out, however, that the diversity is a reflection of the "diverse aspects of the problem", and not necessarily a "diversity of opinions". Spengler discussed policies, which had their positive sides; I was talking about the excution of some of the policies, which led to intense suffering by some people.

My report was written shortly after my return when the depressing discoveries of the suffering by my friends and relatives were still fresh in my memory. If I had been more emotional than I need to be, my motives could be understood, if not forgiven.

Finally, I inadvertently used the expression "tradition-bound arrogance" when I really meant to say "tradition-bound smugness". I am sorry for the unfortunate choice of a word that has been offensive, when no offence was intended.

Yours faithfully,

KEN HSÜ

ETH, Zürich, Switzerland

New select committee seeks expertise

SIR, — The Education, Science and Arts Committee held their first meeting on Thursday, 29 November.

In recommending the setting up of the new select committees, the Procedure Committee stated, "We hope that, as the new committees become established . . . they will develop methods of work which will enable them to respond more speedily to current problems and to new policy proposals". In view of this we have decided to study a number of subjects and report fairly rapidly during the coming year. By fairly rapidly we mean we are trying to set ourselves a maximum time of two months between starting to take evidence and reporting. We have facilities to hire specialist assistance. We have not yet decided which shall be our first subjects of study but we expect them to be in the field of education.

The Committee are anxious to ascertain what specialist assistance might be available to them in their investigations, and I have therefore written to a number of institutions asking if and how they are able to help. But the committee would also like to hear from any individuals and other organisations who feel they have the individuals and other organisations who feel they have the necessary expertise, and I am therefore seeking the hospitality of your columns to make this request.

Yours faithfully,

CHRISTOPHER PRICE MP

House of Commons, London UK

Research councils: do they pay the real cost?

SIR, — I should like to publicise an unfortunate consequence of the restrictions which grant-awarding bodies such as the Science Research Council place on the use to which their grants may be put. In general the recipient is not allowed to use the money to pay for computing, telephoning, typing, photocopying, page charges for publishing, library facilities, recruitment advertising, removal expenses for staff appointed, or redundancy pay. Instead, the host institution is assumed to be capable of paying for all of these. This is unquestionably no longer true. The result of this is that the more active a department or institution is, the closer it is brought to bankruptcy by its scientific endeavour. I would suggest that small institutions are particularly vulnerable to this. Is it not about time that the research council faced up to their responsibility and paid the *full* cost of a research project? Is it really our government's policy to encourage its academic scientists to lay low and do nothing because the funds provided are not sufficient to cover the *real* cost of their projects?

Yours faithfully,

ALAN D.B. MALCOLM

St Mary's Hospital Medical School, London UK

X as the Y of Z

SIR, — In your review of Horace Judson's *The Eighth Day of Creation* (11 October, page 506) I am called "a sort of Louella Parsons of molecular biology" apparently, because I told Judson that I thought a friend of mine is its Douanier Rousseau (a painter whom I very much admire). Here your reviewer is not far off the mark: long ago I picked up the habit of styling X as the Y of Z at a party in Hollywood, where I heard Christopher Isherwood say that Wagner is the Puccini of music.

Before finally kicking the habit, may I coin one final simile? Namely, if *The Eighth Day of Creation* really showed as much lack of historical comprehension as your reviewer found it to show, then Judson would be the Thomas Jukes of molecular biology.

Yours faithfully,

GUNTHER S. STENT

University of California, Berkeley, US.

news and views

Mutants in a mosaic gene reveal functions for introns

from Bernard Dujon

Two years ago molecular geneticists discovered an unexpected organisation for eukaryotic genes: the DNA sequences that specify the primary structure of the gene product (often called exons[1]) are interrupted by other base sequences (called introns or intervening sequences). The origin and the significance of the latter remain a puzzle (see ref. 2). However, one might hypothesise that the different alternating pieces of the same gene, the introns and the exons, could perform different genetic functions recognisable with the help of specific mutations. A mitochondrial gene of the baker's yeast seems to provide an example of this.

After mutations in the mitochondrial gene for cytochrome b, an enzyme of the respiratory chain, were first reported about three years ago[3-5], it soon became apparent that they result in a wide diversity of phenotypes[6, 7]. Some of them are even pleiotropic and also block the synthesis of the subunit 1 of cytochrome oxidase, another element of the respiratory chain whose gene is in another part of the mitochondrial genome. By a combination of genetic and molecular methods[8-11,16] and by a laudable collaborative effort between different groups of investigators, all the mutants (around 200) are now ordered in a unique map[12].

The idea that the cytochrome b gene is a mosaic[13] of, at least, five exons interspersed with four introns is suggested by the following lines of evidence. (1) There is a consensus that the gene product is the 30,000 molecular weight polypeptide, from specific labelling of the mitochondrial translation products[10,14-16]. However, mutations affecting cytochrome b span a region of the mitochondrial DNA which is several times larger than needed to code for this polypeptide and are highly clustered (refs 9, 11, 12 and Fig. 1). (2) The smallest transcript found in this region of the map (2.3 kilobases) and believed to be the mature messenger RNA for the cytochrome b[20-22], hybridises with non-contiguous segments of DNA[20] whose positions and sizes are consistent with expectations from the genetic and physical map. (3) With some mutants the 30,000 molecular weight polypeptide is replaced by a single new polypeptide usually smaller than the wild type[14, 16, 18] as if premature chain termination had occurred. Colinearity[13] between the size of the new polypeptide synthesised and the location of the mutants on the map strongly suggests a single translation unit. This is confirmed in double mutants by an epistatic effect of the upstream mutation on the downstream one[18,19]. (4) With other mutants, interspersed among the first ones, a series of new polypeptides appears in the range of about 15,000 to 60,000 molecular weight[14, 16, 18], suggesting that translation now proceeds also through sequences not normally translated. Peptide mapping confirms that for most cases the new polypeptides do share sequence homologies with the wild type protein[16, 17].

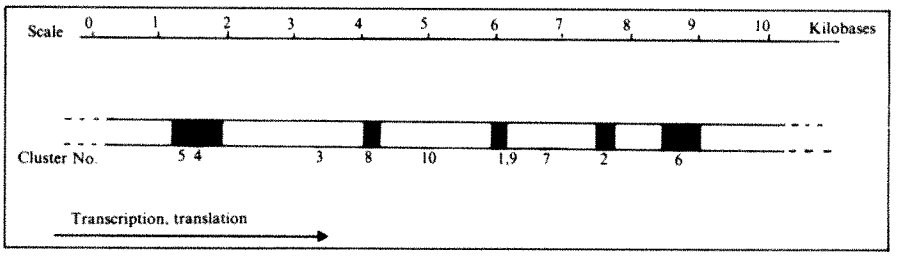


Fig. 1 The cytochrome b gene is a mosaic of at least five exons (black) interspersed with four introns (white). Mutants can be found in both (cluster No). They have been mapped as indicated by a combination of genetic and molecular methods.

A unique contribution of the yeast cytochrome b gene to the more general question of the expression and regulation of eukaryotic split genes lies in one's ability to perform functional complementation tests between mutants. On the basis of this test the mutants fall into two classes[9, 19]. The first is composed of mutants that fail to complement one another (they belong to the clusters 5, 4, 8, 9, 1, 2 and 6; numbering is historical and not logical). Those therefore are satisfactorily explained by the classical notion of cistron and they are called here 'exon mutants'. The idea that they are mutants in exons is confirmed by the fact that drug resistant mutations, affecting the sensitivity of the protein to specific inhibitors but not its activity, are closely linked to them[23, 24]. Now, most 'exon mutants' can be complemented in *trans* by the mutants of the second class (clusters 3, 10 and 7). Thus, mutants of the second class, although interspersed among those of the first, do not belong to the same cistron. Instead, each cluster constitutes a new complementation group. In a mosaic gene the classical notion of the cistron must therefore be accommodated with the fact that some mutations in the continuity of a given cistron will not belong to that cistron, as if different mutants affected different types of functions. Mutants of the second class are described in the literature as 'intron mutants'. Such a definition only refers to the functions altered in the mutants. The location of these mutants within long introns can eventually be established by molecular mapping[9,11]. These mutants therefore suggest a specific role for introns. Interestingly, they show the pleiotropic effect on cytochrome oxidase.

The next refinement in complementation studies was achieved by experiments in which the exons of the two parents are distinguished from each other by the use of the drug resistant mutations as genetic markers. From a series of such experiments performed recently[25] it became clear that the 'exon mutants' can provide in *trans* a function that helps overcome the deficiency(ies) of the 'intron mutants', such that the final gene product can be made using the non-mutated exon sequences of the 'intron mutants'. Consistent with the fact that 'exon mutants' are a unique cistron, all the exons are now always expressed in *cis*. This is diagrammatically shown in Fig. 2. Similarly different 'intron mutants', belonging to two different clusters, can help each other such that both will now be able to express normally their non-mutated exon sequences.

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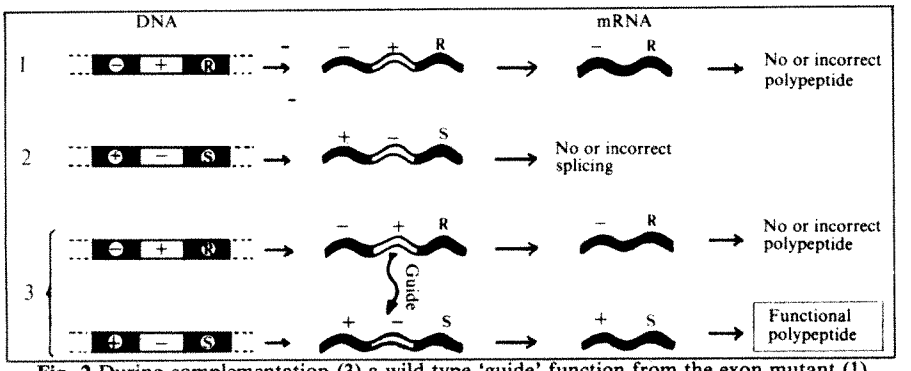


Fig. 2 During complementation (3) a wild type 'guide' function from the exon mutant (1) helps overcome the splicing deficiency of an intron mutant (2) whose non-mutated exon sequences are then used to direct the synthesis of the polypeptide. The 'guide' step is arbitrarily shown as an interaction between two mRNA precursors but could be otherwise.

All the properties of 'intron mutants' reflect a deficiency in a gene function, different from the coding of the primary sequence of the protein, and required at an earlier step than translation. From this we hypothesise a deficiency of RNA splicing. This is directly confirmed by examination of the RNA transcripts showing that 'intron mutants' are actually blocked at different specific steps of splicing[21,22]. Correct RNA splicing therefore would require wild type sequences which, when mutated in a given gene, can be complemented in *trans* by products of another copy of the same gene. This prompted P. Slonimski to propose a new element for gene expression called 'guide RNA'[21,26], whose role is to align the splice junctions or to pull out the skein of messenger RNA precursor for proper splicing to occur. The guide concept is based on the *in vivo* complementation test and is necessary to explain the results. The idea that such a guide role is played by an RNA molecule, and not a protein, is not yet directly demonstrated. But it opens the possibility that successive processing of the different introns could serve as a mechanism for regulating gene expression. Such schemes are now being proposed[21,22].

In those the completion of one step requires the success of a previous step, as if proper splicing of one intron created the guide needed for the splicing of another. The same scheme applies to explain the pleiotropic effect of some 'intron mutants' on the cytochrome oxidase gene[21].

If such a guide exists one would like to know the nature of the sequences involved, their relations to introns, exons or junctions and the molecular form of the guide (for example, is it free spliced intron RNA or part of the precursor). Similarly the mechanism of guidance (for example RNA/RNA base pairing or more complex interactions) is a question for investigation. In addition, the interaction of some mutants of the cytochrome oxidase gene on the expression of cytochrome b[21, 27] may also be a very promising avenue for study. One can then reasonably guess that in the near future an ordered regulatory scheme of splicing of these two genes, assigning specific functions to specific sequences, will be available. Now, what in the genome of mitochondria reflects a high degree of specialisation and what a more general pattern for eukaryotic genes, remains as yet unknown. But, in any case, the cytochrome b gene immediately provides functions for introns that are directly amenable to experimental analysis. □

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Flight formations in geese and other birds

from Robert M. May

AMONG the autumn sights around Princeton are the V-shaped formations of Canada geese, *Branta canadensis*, honking across the sky in flights of up to 100 birds.

It has often been suggested that the purpose of this tidy formation is to take advantage of aerodynamic efficiencies which can accrue to group flight. Figure 1 (from Lissaman & Shollenberger, *Science* **168**, 1003; 1970) illustrates how these effects come about. As these authors explain, the downdraft under the bird (the downward pointing arrows) derives from the "basic principle of aerodynamics that an object flying by dynamic lift in a fluid gets its lift by creating downward momentum within its span. This applies to fish or fowl, airplane or submarine.". Beyond the wing there is an upwash, which is very intense near the wingtip and which is associated with conservation of momentum in the fluid. The resulting streamlines are as depicted in the left half of Fig. 1; these streamlines show the direction of airflow generated by the passage of the bird, and we see they have a general tendency to produce an updraft to the right and left of the bird, and a downdraft above and below it. Thus birds flying side-by-side can take advantage of the updrafts from neighbours, an effect similiar to flying in an upcurrent, with a consequent reduction in the 'lift power' needed. Conversely, birds flying in a vertical column would each need to generate additional lift to compensate for the downdraft effect.

In a quantitative analysis of the magnitude of these effects, Lissaman and Shollenberger have shown that a formation of around 25 or more birds can reduce the power required for 'lift' by a factor of 2.9 by flying wingtip-to-wingtip in a horizontal line. For a given power output, the increase in range scales as the square root of this lift-reduction factor; thus flight formations of 25 or so birds can have a range roughly 70% greater than that of a lone bird. The associated optimum cruising speed decreases as the inverse fourth root of the lift-reduction factor, corresponding to a 24% reduction in the speed of the formation compared with a lone bird. These calculations take into account the effects of lift (or, in aeronautical jargon, 'induced drag') and of the power needed to overcome direct air resistance ('profile drag'), but they ignore the complications of flapping wings. The flapping losses are likely to be negligible so long as the wingtip flapping speed is significantly less than the

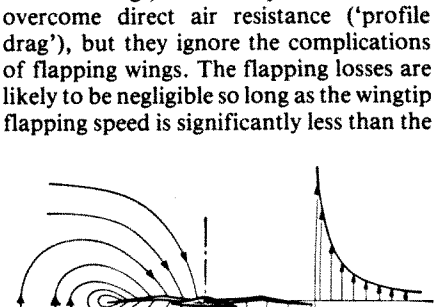


Fig. 1 Flow field associated with a flying bird.

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forward flight speed; this is so for geese, but is not always true for the passerines discussed below.

The basic calculation of Lissaman and Shollenberger is for a horizontal formation of birds advancing in line abreast. But 'Munk's stagger theorem' states that in a classical fluid (where circulation is conserved) any member of an assembly of lifting surfaces may be translated in the direction of its flight path without changing the total induced drag. Now, in the line abreast formation the end birds enjoy relatively less, and the centre birds relatively more, of the upwash; indeed, with more than ten birds, the central birds have double the savings of the end ones. By staggering the formation into a V, the induced drag can be shared evenly, without affecting the total for the formation. The V does this by virtue of the lead bird experiencing a weaker upwash field even though it is mainly on one side. In detail, the 'optimum' configuration is like a slightly rounded V, with a blunted point and more acutely trailing tips. For a formation of nine birds, Lissaman and Shollenberger's calculations suggest the angle of the V to be roughly 120°. The formation has, moreover, a kind of stability in that a bird forging ahead will tend (under constant power) to fall back, and a bird dropping back will tend (under constant power) to catch up; more subtly, the formation is not stable against a bird which drops back and reduces power.



Canada Geese flying

Gould and Heppner (*Auk* **91**, 494; 1974) have attempted to test these ideas, by analysing films showing formations of Canada geese in North America. They made observations of more than 100 flights of flocks, averaging around 27 geese. The first disconcerting observation was that over 40% of the formations were echelons, wherein the lead bird must enjoy significantly less than the average updraft. Moreover, in the five cases where the data yielded a steady result, they found (after correcting for the distortions created by viewing the formation from the ground and at an oblique angle) an average V-angle of 35°. Gould and Heppner also find other V-angles of 31° and 36° mentioned in the literature, by people who chanced to see flocks directly overhead. They also report unpublished radar observations by Williams, Klonowski and Berkeley, which give an average V-angle of 71°. (The radar data were collected in the spring, the others in the autumn, and in all cases the geese were flying from feeding to roosting grounds, rather than being in long-distance migration.) In short, almost half the formations were echelons, and those that were V-formations had angles consistently much less than Lissaman and Shollenberger predicted on the basis of equalisation of uplift among the birds.

Gould and Heppner conclude that aerodynamic effects are not the main reason for V-formations, and suggest other explanations (such as the birds keeping each other in view). I think this conclusion is too strong. Another piece of the information elegantly gathered by Gould and Heppner is the spacing of birds along the arms of the V, which ranges from 3.2 m to 4.7 m for different V-angles. The key distance, however, is this spacing projected along the line of flight (that is, the equivalent line-abreast spacing), which averages about 1 — 1.5 m, or around the wingtip-to-wingtip distance (approximately 1.5m) that gives maximum overall lift. This arguably is the main phenomenon, and its total magnitude is (according to Munk's stagger theorem) independent of whether the flock flies in line abreast, in echelon, or in a V of arbitrary angle. The question of equalising the advantage among the birds is a much subtler one, and evokes echoes of similar questions about the "geometry of the selfish herd" (Hamilton, *J. theor. Biol.* **31**, 295; 1971), where aggregation does confer some overall benefits on the herd, but where at any one moment these benefits are enjoyed more by the inner than by the outer members. It would be interesting to know if the disadvantaged lead goose keeps the lead for long periods, or whether the lead continually shifts.

The preceding discussion pertains to horizontal line formations, as are found in geese. But many other species of birds conduct their long twice-yearly migrations in large three-dimensional flocks. This is the case for most migratory passerines. Higdon and Corrsin (*Am. Nat.* **112**, 727; 1978) have analysed the net aerodynamic advantage associated with such flock-flying, as a function of the overall shape of the flock. If the average separation between birds is reasonably large compared to the wingspan of an individual bird, an elegant nineteenth-century-tripos-style calculation is possible, regarding each bird as a dipole vortex and summing over all birds. For a two-dimensional flock of such birds, randomly arranged within a vertical ellipse of height B and width A, the flock-associated decrease in induced drag (or upwash) on each bird, expressed as a ratio to the induced drag in sole flight is

$$(\pi^3/64)nd^2(A-B)/(A+B). \qquad (1)$$

Here n is the average number of birds per unit area and d is the wingspan; thus nd^2 can be rewritten as $(d/\Delta)^2$, where Δ is the average spacing between birds. (The expression (1) differs from Higdon and Corrsin's by an unimportant numerical factor of 4, which they lose in the process of making a sequence of unnecessary approximations.) The maximum aerodynamic advantage is obtained when the horizontal extension of the flock greatly exceeds the vertical ($A \gg B$), whereas if the vertical dimension exceeds the horizontal one ($B > A$), the net effect is an increased downdraft. This general observation does not depend on the detailed shape of the flock, and it follows broadly from the earlier discussion

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associated with Fig. 1. We can see that, unless the birds fly closer together than observations suggest, the aerodynamic effects of such flight in two-dimensional flocks is slight; even if the spacing between birds is as little as twice the wingspan ($\Delta \sim 2d$), the uplift effect in a horizontally extended flock is only around 10%. Higdon and Corrsin estimate the corrections to equation (1), in the event of the birds flying in some ordered lattice pattern, rather than more-or-less randomly. These correction terms are of order n^2d^4; that is, they are smaller than those given in equation (1), by a factor of order $(d/\Delta)^2$.

Extending the analysis to encompass a fully three-dimensional flock is in principle straightforward (by virtue, again, of Munk's stagger theorem, which enables the three-dimensional problem to be projected into a two-dimensional one). In a randomly arranged flock, the upshot will be to regain the expression (1), but with n now being the average number of birds projected onto unit area perpendicular to the flight direction. Clearly, the result will be to increase the uplift effect (1) as n increases, provided the flock is extended more horizontally than vertically. If the effective value of n becomes too large (so that nd^2 is not appreciably less than unity), the 'dipole vortex' approximation will begin to break down, and the computations must be based on pairs of vortices centred at a bird's two wingtips. But for most three-dimensional flocks of migratory birds the density is unlikely to make this refinement necessary. I take home the message that three-dimensional flocks of passerines and other birds can gain non-negligible updraft effects, provided the flock is extended more horizontally than vertically, but that the magnitude of the effect is not nearly as large as that gained in the close, horizontal line formation of geese.

A different kind of insight into the dynamics and ergonomics of migratory flight comes from Allerstam (*J. theor. Biol.* **79**, 341; 1979). Starting with the observation that wind speed is usually less at low than at high altitude, he examines the idea that migratory birds may save flying time and energy by allowing themselves partly to be drifted by strong winds at high altitude, and then correcting their course at low altitude in relatively weaker winds. For typical values of wind velocities, Allerstam's calculations suggest this behaviour will be advantageous if the strong upper winds are blowing in a direction roughly 30° to 90° wide of the goal direction ('following side winds'). If the wind is blowing at an angle less than 30° to the desired direction, a direct high altitude flight is optimal. Conversely, a direct low altitude flight is the best route against a headwind (in excess of 90° wide of the goal direction). Allerstam concludes with a survey of the available evidence, of which the best (Gruys-Casimir, *Arch. Neerl. Zool.* **16**, 175; 1965) is on chaffinch migration in Holland: "Radar observations of drift in high altitude bird migration and visual records of low altitude overcompensation are compatible with the optimal flight behaviour of migrants at high and low altitude, respectively, as predicted from this hypothesis." □

Mycorrhizal associations

from Peter D. Moore

MYCORRHIZAL associations between plant roots and fungi are now known to be extremely common in nature. It is evident that such associations are symbiotic, both parties benefitting from the interaction. As far as the plant is concerned, the association may be very costly in terms of the loss of fixed carbon to its heterotrophic partner. Lewis and Harley (*New Phytol.* **64**, 256; 1965) fed ^{14}C sucrose to cut roots of beech (*Fagus sylvatica*) and observed that 50–75% of the carbon reaching the root tip was passed on to the mycrorrhizal fungus, which obviously represents a considerable drain on the photosynthetic resources of the plant.

The widespread occurrence of mycorrhizae, however, witnesses to the evolutionary value of the partnership to host plant as well as fungus. It has long been established that a major advantage to the plant is the greater efficiency with which it is able to absorb necessary inorganic materials from the soil (Harley *J. Ecol.* **59**, 653; 1971). There is also some evidence to suggest that water uptake is assisted by mycorrhizal infection (Safir *et al. Plant Physiol.* **49**, 700; 1972). This being so, one might expect mycorrhizal associations to be of particular value, and therefore especially prevalent, in habitats deficient in plant nutrients and water.

Grime (*Plant Strategies and Vegetation Processes*, 36; Wiley, Chichester, New York, 1979) takes up this argument in his discussion of stress-tolerant plant species. Grime proposes an ecological classification of plants into competitors, stress-tolerators and ruderals, and he claims that mycorrhizal associations, particularly of an ectotrophic type, are to be expected amongst plant species adapated to nutrient stresses. Data recently published deriving from arid regions of the United States support and extend this view.

Reeves, Wagner, Moorman and Kiel (*Am. J. Bot.* **66**, 6; 1979) have conducted a survey of vesicular-arbuscular (endo-) mycorrhizae in semi-arid sagebrush communities of Colorado. Of the 42 species of plant present, only 2 were found to lack mycorrhizal infection, one chenopod and one crucifer. This emphasises the widespread nature of mycorrhizae in a taxonomically diverse flora: 13 families are represented and all but one have mycorrhizal members present.

The authors also investigated a nearby site which had been severely disturbed by road construction. Here 21 plant species were growing, but only 6 possessed mycorrhizal infections; these species had also been present in the undisturbed site. The remaining 15 species were confined to the region of disturbance and had no detectable mycorrhizal infection (three individuals of each species were examined). Thus, in an unstable habitat, the mycorrhizal plants could be at a disadvantage, perhaps because of the destruction of a large proportion of the residual fungus in the soil during the process of disturbance which might restrict recolonisation by mycorrhizal species of plants. The authors propose that early successional stages are deficient in mycorrhizal species, whereas in later, more stable stages the vast majority of species are so adapted. This seems to be justified for their arid, nutrient-poor habitat.

The lack of mycorrhizal species in disturbed sites is confirmed by the results of a similar investigation by Miller (*Can. J. Bot.* **57**, 619; 1979) of mine spoil heaps and nearby undisturbed sage scrub in Wyoming. In these latter, pristine areas, 21 species were examined and only one lacked vesicular-arbuscular mycorrhizal infection. On the spoil heaps 16 species were found, none of which was infected. Even within one family, the Chenopodiaceae, some members are non-mycorrhizal and ruderal in habit, whereas others (the majority) are mycorrhizal and are found in undisturbed habitats where edaphic stresses of nutrient and water availability are encountered.

So the Grime hypothesis seems to hold true for these semi-arid ecosystems: there does appear to be a division among plants which corresponds to his stress-tolerant and ruderal types. What is not clear from these particular sites is the outcome of low disturbance and low stress. In other words, of what importance are endomycorrhizae to the Grime competitor? □

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100 years ago

M.A. Guyard claims to have discovered another new metal of the platinum group which he names uralium, from the Ural Mountains, whence the ore is procured. There have been quite a flood of similar announcements lately. We have now gallium, davyum, mosandrum, neptunium, decipium, phillipium, norvegium, scandium, ytterbium, holmium, "X", thulium, and uralium. Chemists will have to keep as narrow a watch on these minor elements as our astronomers do upon the minor planets, or we shall not know where we are.

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The transforming genes of SV40 and polyoma

from Peter Rigby

SIMIAN virus 40 (SV40) and its relative polyoma (which naturally infects mice) are small DNA viruses which have relatively simple genomes that provide attractive and easily manipulated systems for probing the mechanisms of eukaryotic gene expression. They have also attracted great interest because of their ability to transform some mammalian cells *in vitro*. Such transformed cells have many of the properties of tumour cells and have been studied in the hope of elucidating the mechanisms by which viral gene expression can over-ride the normal controls on cell growth and behaviour. During the past few years great progress has been made in defining the viral genes and their products responsible for transformation. It has been known for some time that the so-called 'early' region (those genes expressed soon after infection and before viral DNA replication in cells in which the virus is able to multiply) is sufficient to induce transformation. Work on the transforming capacity of SV40 and polyoma has therefore focused on defining the products of the early region, and recently published work, together with results presented at two meetings* last summer, have greatly increased our understanding of the structure of this region and of the properties of its protein products.

The availability of complete DNA sequences for both SV40 and polyoma[1-8] has enabled the relationship between the various protein products of the early region, classically identified by immunoprecipitation with sera from animals bearing virus-induced tumours, to be clarified. Originally only one such SV40 'tumour' antigen (T-antigen) was recognised. This is a protein of apparent molecular weight 100,000, now called large T-antigen; a protein of this size would account for almost all the coding capacity of the early region. Prives *et al.*[9] then showed that among the products of *in vitro* translation of viral mRNA obtained from infected cells was another immunoprecipitable protein of molecular weight 17,000 — the small t-antigen.

Large T/small t

The first clue to the origin of small t came with the discovery of viable SV40 deletion mutants that produce normal large T despite the fact that the deletions are within the sequence that was thought to code for this protein[10]. However, these mutants do not induce the synthesis of full size small t[11] and transform cells at a reduced frequency under certain assay conditions[12,13]. At about the same time Berk and Sharp[14] showed that there were two SV40 early mRNAs and not one as had previously been thought. These mRNAs have the same 5′ and 3′ ends but are spliced differently (see Fig. 1). The smaller of the two early mRNAs encodes large T[15]; the sequences missing in the viable early deletion mutants correspond to those normally spliced out of this mRNA and so the protein is unaffected. In the other mRNA fewer nucleotides are removed by splicing and the additional mRNA sequences contain an in-phase termination codon. Thus this message directs the synthesis of small t[15]. The relationship between the two tumour antigens shown in Fig. 1 has been confirmed by peptide mapping and sequencing studies[16-19].

The analogous polyoma mutants, known as *hr-t* (host range-transformation) mutants, were isolated many years ago by Benjamin[20] and are absolutely defective for transformation. *hr-t* mutations, which can be either deletions or point mutations, map in the region of the polyoma genome analogous to that deleted in the viable early deletion mutants of SV40[21,22] and it was shown that polyoma also encodes a small t which is affected by *hr-t* mutations although the large T remains normal[23,24].

Polyoma middle T-antigen

Immunoprecipitation of extracts of polyoma-infected and transformed cells with anti-tumour serum has identified a third tumour antigen, of molecular weight 55,000 — middle T-antigen[25]. This is found in association with the plasma membrane and probably has the same amino-terminal sequence as small t and large T[26-28]. But peptide maps show that middle T contains peptides found in neither of the other two tumour antigens suggesting either that it is translated in a different reading frame or that it is partially coded by the host cell[26,27]. However, the mRNA for middle T hybridises specifically to polyoma DNA[29], this protein as well as small t is altered by *hr-t* deletions[24,28] and examination of the polyoma DNA sequence reveals the presence of a second open reading frame in the appropriate part of the early region.

SV40-transformed cells contain an immunoprecipitable protein of similar molecular weight, 53,000, and it was initially thought that this might be the analogue of polyoma middle T. The relevant region of the SV40 genome, however, contains only one open reading frame, that used for large T, and it is now clear that this protein, which is discussed in more detail below, is of cellular origin.

Organisation of integrated viral DNA

In cells transformed by SV40 or polyoma, viral DNA is covalently integrated into the chromosomal DNA of the host cell and the expression of this viral information is responsible for the transformed phenotype. There is a great deal of new information on the organisation and expression of these integrated sequences which is helping to identify the roles of the different tumour antigens in transformation. The organisation of the integrated viral DNA in many transformed mouse and rat cell lines has been analysed by restriction mapping. The general conclusion is that integration is not specific, each line has a characteristic pattern of virus-specific DNA fragments indicating that many different sites on both viral and cellular DNAs can be used for integrative recombination[30-33]. However, F. Cuzin (Nice) has reported that identical integration patterns are observed in two sets of SV40-transformed cells, one set of three rat lines the other of two mouse lines. There are distinct biological differences between the three rat lines but in view of the preponderance of data suggesting random integration the significance of these observations is unclear.

The structure of the integrated viral DNA can provide clues to possible mechanisms of integration. A high proportion of SV40 and polyoma-transformed cell lines carry tandem duplications of all or part of the viral genome[32,33]. Such structures would not be expected if recombination occurs between monomeric, circular viral DNA and linear chromosomal DNA. However, P. Rigby (Imperial College, London) discussed data indicating that SV40 DNA replicates in mouse cells to produce large, tandemly-arrayed polymers of viral DNA and that these polymers recombine effectively with one another. If such polymers are the precursors of the integrated form the high frequency of tandemly-duplicted insertions would be explained. When some transformed cells are fused with permissive cells infectious virus can be rescued and in certain cases the DNA of the rescued virus is identical in structure to that of the virus used to transform the cells. Such perfect excision may well result from the presence of tandemly-duplicated insertions and the loss of a complete copy of the viral genome from a tandem array has been demonstrated in some polyoma-transformed lines[34]. However, excision does not require tandem duplication; when the transformed rat cell line 14B, which carries a defective copy of the viral genome, is fused with permissive cells imperfect excision occurs giving rise to a heterogeneous population of circular molecules containing both viral and cellular sequences (M. Botchan, Cold Spring Harbor Laboratory, New York). Some of these rescued DNAs have been cloned and sequence analysis shows that the structure of the viral-cellular joins is unaltered. It has been proposed that this type of excision may proceed by way of *in situ* replication of the integrated viral

*XLIV Cold Spring Harbor Symposium on Quantitative Biology 'Viral Oncogenes', 30 May-6 June 1979; and the ICRF 1979 Tumour Virus Meeting, 16-20 July, 1979, Cambridge.

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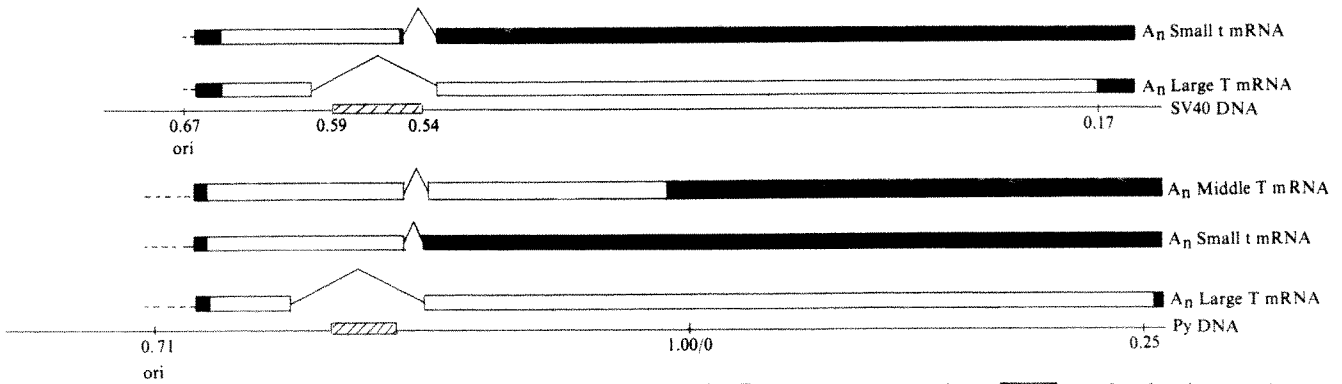


Fig. 1 mRNAs found in cells productively infected by SV40 or polyoma. ▬, Non-translated regions; ▭ translated regions; ⌒, splices; ▨, regions of DNA deleted in viable early deletion mutants (SV40) or *hr-t*mutants (Py); - - - -, 5 -end heterogeneities.

sequences rather than by a recombinational excision[35].

J. Sambrook and J. Stringer (Cold Spring Harbor Laboratory, New York) described the cloning of the single integrated SV40 genomes of two transformed rat cell lines. In one line, 14B, the integrated sequences are not colinear with free viral DNA; one segment of the genome has been deleted while another is inverted, giving a structure that Sambrook suggested could be derived from recombination between cellular DNA and a replicative intermediate of viral DNA. The cellular-viral DNA junctions in this line have been sequenced; there is no detectable homology between the viral DNA and the cellular DNA into which it is integrated suggesting that integration occurs by illegitimate recombination. The cellular sequences flanking the integrated viral DNA have been compared in detail to those flanking another integrated viral genome that has been cloned, from line 17, and there are no detectable similarities or sequence homology.

T antigens and transformation

Although there is much evidence to implicate large T in transformation by SV40, elucidation of the structure of the integrated viral DNA in polyoma-transformed cell lines suggests that polyoma large T is not essential for transformation. Some polyoma-transformed cell lines do not carry complete copies of the early region; although such lines may have tandemly duplicated insertions each copy of the early region is interrupted, either by a deletion or by a virus-host junction, in such a way that there is no complete coding sequence for large T (M. Fried & L. Lania, ICRF, London). Analyses of the mRNAs (R. Kamen, ICRF) and of the tumour antigens present in such lines confirm that they contain small t and middle T but not large T, although in some lines truncated derivatives of large T are present.

More direct evidence that polyoma transformation does not necessarily require intact large T is provided by experiments in which viral DNA, either intact or cleaved by restriction endonucleases, is injected into newborn hamsters[36]. If the DNA is cleaved to interrupt the early region coding sequences unique to large T, tumorigenicity is increased over that of supercoiled intact DNA and cell lines established from the resultant tumours contain only small t and middle T. It might be argued that large T is produced from uncleaved or recircularised DNA during the initial stages of tumour induction. This possibility has been eliminated by experiments in which hamsters are injected with a recombinant plasmid containing polyoma DNA cloned through its *Eco*RI site, which lies within the unique large T coding sequence, into the *Eco*RI site of pBR322 (M. Martin & M. Israel, National Institutes of Health, Bethesda). Tumours were obtained and cell lines established from them. Such lines lack large T and analysis of the DNA of one of them shows that the integrated DNA retains the two polyoma-pBR322 joints. J. Hassell (McGill University, Montreal) reported data showing that cloned fragments of polyoma DNA that contain the coding sequences of small t and middle T, but lack almost all the sequences unique to large T, will transform cells *in vitro* and that the resulting cell lines have a standard transformed phenotype. Further confirmation of the role of the various tumour antigens in polyoma transformation is provided by an experiment in which rat cells were infected with an *hr-t* mutant and cell lines were established from clones picked at random[37]. One such line contains the genome of the *hr-t* mutant in both free and integrated forms. The cells synthesise large T but not small t nor middle T; they have a normal phenotype *in vitro* and will not induce tumours *in vivo*, showing that large T itself is insufficient to maintain the transformed state. Mutants of polyoma with deletions in the region coding for both middle T and large T have altered transformation properties[38] and some of the cell lines that are isolated do not have all the characteristics of a fully transformed cell (B. Griffin, ICRF). When taken together these data imply that only half of the early region is required to initiate and maintain transformation and suggest that middle T may be the most important protein for this process.

Temperature-sensitive polyoma mutants of the *ts-a* class map in the region coding uniquely for large T[39,40] and it was shown many years ago that, at the non-permissive temperature, such mutants are defective for the initiation but not for the maintenance of transformation[41]. It may well be that in a normal virus infection large T is required to initiate transformation, perhaps because integration requires viral DNA replication, as has been suggested for SV40. Transformation with DNA involves the cells receiving massive doses and in such cases other integration mechanisms, not dependent on DNA replication, may operate. What is clear is that polyoma-transformed cells can retain their phenotype in the absence of intact large T, whereas all SV40-transformed lines examined so far retain and express all of the large T coding sequences. The difference between the two viruses presumably reflects the fact that polyoma has evolved a third early protein, middle T, which SV40 does not have.

Polyoma transcription

The transcription of the early region of polyoma DNA has been studied in great detail by Kamen and his colleagues. Their powerful two-dimensional modification of the Berk and Sharp S1 endonuclease mapping procedures reveals three major mRNAs in productively-infected cells (see Fig. 1) which presumably code for the three tumour antigens. The mRNAs for small t and large T have a similar relationship in both polyoma and SV40. Fitting the S1 mapping data to the DNA sequence indicates that the mRNA for middle T is derived by splicing the leader of small t mRNA to a body that begins some twelve nucleotides downstream of the body of small t mRNA, thus eliminating the termination codon used for small t and effecting the change in reading frame necessary to produce middle T. Polyoma-infected cells also contain a minor class of mRNAs, containing representatives of all three splicing patterns, the 3′ ends of which map close to the middle of the early region, just past the termination codon for middle T.

In polyoma-transformed rat and mouse cells the 5′ ends of the mRNAs correspond

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to those found early in productively-infected cells, suggesting that transcription is initiated at the viral rather than a host cell promoter. Almost all transformed cell lines overproduce the shortened mRNAs that have polyadenylated 3′ ends mapping in the middle of the early region and in one mouse line the vast majority of the viral mRNA terminates at this site, although the virus-host junction in the integrated DNA is 0.75 kilobases further downstream. There has been much speculation regarding the existence in transformed cells of fused virus-host transcripts. The integrated viral DNA from one mouse line, *ts-a*3T3-3, has been cloned in λ (Fried) and Kamen's group have mapped the mRNAs against this isolated DNA segment. Their data show clearly that the polyadenylated ends lie some 60 nucleotides into the cellular DNA at the 3′ end of the transcription unit, thus providing the first definitive evidence for fused transcripts.

SV40 transcription

Transcription in SV40-transformed cells has not yet been analysed in the same detail. Sambrook reported that the mRNAs of the rat lines 14B and 17 had been mapped against the cloned integrated genomes using the S1 endonuclease method; they seem to be identical to the two early transcripts found in productively-infected cells. However, M. Lovett (Imperial College, London) presented transfer hybridisation experiments[42] showing that in several mouse lines novel transcripts, both larger and smaller than those found in infected cells, can be detected. One line contains derivatives of both small t and large T mRNAs that extend past the normal viral polyadenylation signal but it is not clear whether they terminate at a viral or a cellular site. E. May (IRSC, Villejuif) discussed S1 mapping data suggesting that some rat lines synthesise mRNAs which contain tandem duplications of viral sequences coding for large T. *In vitro* translation of such transcripts shows that they encode very large proteins related to large T; these super T-antigens are quite widespread[43-45]. Peptide mapping indicates that in some cases super T proteins contain peptides from the C-terminal half of large T in more than one molar yield (F. Chaudry, ICRF) suggesting that the amino acid sequence is also tandemly duplicated.

A cellular protein associated with T-antigens in SV40-transformed cells

As well as the virus-coded T-antigens SV40-transformed cells contain an immunoprecipitable protein of molecular weight 53,000, the so-called nvT-antigen (non-viral T-antigen). Peptide analysis shows that this protein is unrelated to small t or large T and that it is different in transformed cells of different species[43-45]. It has also been found in uninfected mouse 3T3 cells and undifferentiated mouse teratocarcinoma cells[46,47]. Its synthesis is induced by SV40 infection and this induction requires the activity of large T[47]. In extracts of transformed cells nvT cosediments with the polymeric, highly-phosphorylated form of large T (F. McCormick, ICRF). These experiments confirm the proposition, first advanced by Lane and Crawford on the basis of immunological experiments[48], that nvT is precipitated by anti-tumour serum because it exists intracellularly as a complex with large T. G. Jay and E. Appella (NIH) reported that a 53,000 molecular weight protein, which may be identical to nvT, is recognised by antisera raised against methylcholanthrene-induced mouse sarcomas and that this protein is expressed in cells transformed by many agents including SV40. It is found in chemically-induced sarcomas, leukaemias induced by either X-irradiation or Moloney murine Leukaemia virus, in cells transformed by the Moloney or Kirsten strains of murine sarcoma virus and in spontaneously-transformed fibroblasts. The protein is also detectable in undifferentiated F-9 teratocarcinoma cells but not in normal 3T3 cells, primary embryo cells, adult lung fibroblasts, haematopoietic cells or lymphoid cells. Most interestingly the protein is present in normal thymus cells which have an extremely high mitotic rate and it appears to be synthesised only at particular stages of the cell cycle. It is tempting to speculate that nvT is involved in the control of cellular DNA replication and that complex formation between it and large T is part of the mechanism by which the virus over-rides the normal control of cellular DNA replication.

T antigens as enzymes

The enzymatic function(s) of the papovavirus T-antigens continues to be somewhat controversial. Following the demonstration that the transforming protein of the RNA tumour virus Rous sarcoma virus (RSV) has protein kinase activity[49] several groups reported that SV40 large T has a similar activity[50,51]. However, R. Tjian (University of California, Berkeley), working with the hybrid protein encoded by the adenovirus-SV40 hybrid D2, now claims that the kinase activity can be separated from the D2 T-antigen by purification procedures which do not separate the ATPase activity also associated with this protein. Glycerol gradient centrifugation experiments show that the ATPase binds well to SV40 DNA, a property expected of an activity of large T which binds to the viral origin of DNA replication[52,53], whereas the kinase binds rather poorly. Tjian has now isolated large T from SV40-infected monkey cells; although it has not been purified as extensively as the D2 protein, 99.9% of the kinase activity can be separated from the large T is two chromatographic steps. Moreover, large T isolated from cells infected with *tsA* mutants has a thermolabile ATPase activity but the removed kinase is thermostable. L. Hager (University of Illinois, Urbana) also reported work indicating that large T has an ATPase activity that is stimulated by oligo(dT) but that it is not a kinase.

However, D. Livingston (Harvard University) presented data indicating that highly purified preparations of large T from transformed human cells do have a kinase activity and that the protein isolated from cells infected with *tsA* mutants is temperature-sensitive for the transfer of phosphate to casein. The situation remains confused but it seems that the identification of SV40 large T as a protein kinase is somewhat suspect. It may be unreasonable to expect the enzymatic activities of the transforming proteins of SV40 and RSV to be the same, particularly as large T is predominantly nuclear whereas the *src* protein is located on the plasma membrane[54].

None of the polyoma tumour antigens has been purified but there are suggestions that middle T, which is found in the plasma membrane, is at least associated with a kinase activity (W. Eckhart, Salk Institute; B. Schaffhausen, Harvard University; A. Smith, ICRF). This work relies on a kinase assay in which phosphate is transferred

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from ATP to immunoglobulin or to middle T itself in immunoprecipitates of polyoma tumour antigens; it is thus impossible directly to implicate a virus-coded protein in the enzymatic reaction. However, kinase activity is detected in plasma membrane fractions, the location of middle T, and in transformed cell lines which lack large T but contain middle T. Moreover, there is no kinase activity in the cell line that contains the integrated DNA of an *hr-t* mutant and thus produces large T but not middle T. These results raise the possibility that middle T is a kinase, but they cannot rule out that the enzyme activity is a property of one of the other, less well characterised, viral proteins found in immunoprecipitates, or of a cellular protein that binds specifically to middle T.

In the immediate future we can expect the application of recombinant DNA techniques to lead to a detailed description of the integrated viral DNA found in transformed cells and of the mRNA species synthesised from it. In the longer term the central question to be addressed regarding the molecular biology of SV40 and polyoma will concern the enzymatic activities of the tumour antigens and the cellular targets on which these proteins act.

Ancient plate speeds

from Peter J. Smith

ABSOLUTE speeds of the Earth's lithospheric plates are generally lower for those plates having the greater proportions of continents, higher for those plates having the lengthier subducting edges, and higher for those plates with the greater colatitudes. But can these generalisations be regarded as universal rules holding over all time, or are they merely conclusions temporarily valid under current circumstances? Certainly the statistics are hardly impressive enough to inspire confidence in universality. It is true, for example, that all the plates having large continental components ($>2\times10^7$ km^2) have low speeds (< 30 mm a year); but there are only four of them, or not really enough to support the conclusion that large continents always move slowly. More generally, it would be rash to impute timelessness to any conclusion drawn from the behaviour of the mere 10–12 major plates in existence today.

On the other hand, investigation of past plate speeds is difficult. One approach would be to calculate absolute plate motions on the assumption that the Earth's hot spots are fixed with respect to the mantle; but it is not entirely clear that the assumption is valid, and ancient hot spot traces are difficult to recognise anyway. Solomon *et al.* (*J. geophys. Res.* **82**, 203; 1977) made rather more progress when they estimated plate motions during the early Tertiary on the assumption that there was no net torque between the lithosphere and mesosphere, concluding that generalisations derived from current plate behaviour were not always true in the past. But there is a limit on how far back in time this method can be applied, for it requires complete knowledge of ancient plate boundaries. It is also rather sensitive to the (usually unknown) amount of intraplate deformation which has occurred since.

In view of such difficulties, Gordon *et al.* (*J. geophys. Res.* **84**, 5480; 1979) have now devised a third method of estimating ancient plate motions — one based on palaeomagnetic data. The advantages of such a technique are that palaeomagnetic results for many continents cover at least several hundred million years and that it is possible in principle to determine apparent polar wandering paths with considerable accuracy. The most serious disadvantage, however, is that palaeomagnetism does not give the information necessary to specify continental motion in full. What Gordon and his colleagues have done is to devise a method whereby the data inherent in the polar wandering curve are converted into the rms velocity of the plate — a by no means simple procedure, for the precise relationship of apparent polar wandering to continental drift depends on the position of the continent with respect to the plate's pole of rotation. Because of the fundamental incompleteness of palaeomagnetic data, however, what emerges is not the actual rms velocity of a plate but a minimum rms velocity.

Obviously this is not ideal, but it turns out to be far from useless. Gordon *et al.* have applied their technique to the polar wandering curves for North America, Eurasia and Gondwanaland as specified by Irving (*Nature* **270**, 304; 1977) for the past 350 million years. The results clearly show that the relevant minimum rms continental velocities (V_m) have certainly not remained constant over that period. For North America V_m peaked about 190 million years ago at a value of more than twice the present one, and similar maxima occurred for Gondwanaland a few tens of millions of years earlier and for Eurasia slightly later. Indeed, the peak values of V_m were almost as great as the actual rms velocities of present oceanic plates. In each case the period of particularly rapid motion probably lasted 20–30 million years, which is more than sufficient to indicate that plates with large continents can move rapidly when circumstances permit.

In other words, both largely oceanic and largely continental plates are capable of moving rapidly (that is, at velocities greater than 60 mm a year) — which must mean that the resistance to motion of continental lithosphere cannot be substantially greater than that of oceanic lithosphere. The implication is that the present low velocities of continental plates arise from an accident of plate geometry. Gordon and his coworkers go on to speculate that the chief factor governing plate velocity is the proportion of plate boundary attached to subducting slabs. It just so happens that at present the largely continental plates are short on subduction zones; *ergo*, they are now moving only slowly.

Nevertheless, it may be quite wrong to conclude from all this that at any given time continental plates are just as likely to be travelling as fast as oceanic plates. Continental plates evidently can move as fast as oceanic plates, but the latter probably spend a higher proportion of their time actually moving rapidly than do the former. For although the motional resistances of the two types of plate may be comparable, the subduction cycles of the two may be quite different. Put very briefly, because continental and oceanic lithospheres behave rather differently when approaching and meeting trenches, subduction of an oceanic plate will continue for much longer than subduction of an otherwise comparable continental plate. The result, according to Gordon *et al.*, is that "Statistically . . . plate motions sampled at a given instant of time, such as the present, are likely to show a negative correlation between plate velocity and continental area." □

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100 years ago

There is much more than the name of Cambridge to remind one of its namesake at home. Its quiet air of studious retirement, its quaint buildings and tree-shaded walks have much of the mother-country about them. One or two features of the place, however, are characteristically American. Thus in the great library at Gore Hall, most of the work of receiving and distributing books is done by young women, and done, too, with a noiseless decorum and celerity worthy of all praise. A magnificent Memorial Hall to those graduates of Harvard who fell in the late Civil War bears witness in its crowded lists of names that culture and courage may go hand in hand.

While Harvard is necessarily the great centre of scientific research, much admirable work is done in Boston in the way of practically expounding science. The Institute of Technology has for its primary object the education of the community in these branches of scientific knowledge conducive to progress in the arts and industries of life. In pursuance of this aim the methods of tuition are so practical and thorough that the results must be felt far beyond the industrial circles. Established mainly through the enlightened zeal of the present venerable President of the National Academy, Prof. W. B. Rogers, it began a few years ago to languish, but its founder has recently come back to its rescue, throwing himself into its affairs with all his old heartiness and kindliness until, freshened and stimulated by his influence, it is once more shooting up into lusty vigour.

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articles

Deuterium concentration in the early Solar System: hydrogen and oxygen isotope study

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D/H ratios in the unequilibrated chondrite Chainpur range up to 9×10^{-4}. Corresponding ^{17}O contents are 1‰ higher than in other LL chondrites, with varying ^{18}O contents. These results are discussed in terms of low temperature condensation of water and early irradiation by intermediate energy protons. The latter explanation is favoured.

THE determination of universal deuterium abundances is a major limiting factor on the density of the Universe and helps to determine a model for its expansion[1–3]. Several estimates of the D/H ratio in the protosolar nebula have been made on the basis of experimental data[4,5] and theoretical considerations[6,7]. Recent results give the average ratio as $1.9 \pm 1.1 \times 10^{-5}$ (ref. 8). Present theories on stellar evolution suggest that the thermonuclear reactions taking place at the centre of the Sun totally convert the deuterium into ^{3}He. Calculations show that the D/H ratio should be $\sim 10^{-17}$; direct measurements give a value of $< 2.5 \times 10^{-7}$ at the solar surface[9] and $< 3 \times 10^{-6}$ in solar wind[10]. Hence only planets and meteorites could have preserved some trace of deuterium cosmic abundance. Evolution of the primitive atmospheres on terrestrial planets has undoubtedly altered the initial abundance of deuterium during outgassing of the planet and preferential losses of light hydrogen in the upper atmosphere. On the Moon, present nuclear phenomena (implantation of solar wind protons, *in situ* production of spallation deuterium by galactic cosmic rays) are strong enough to blank out any trace of the primordial isotopic ratio.

In 1954, Boato[11] measured the D/H ratios in some type I and II carbonaceous chondrites. His work did not completely determine the amount of terrestrial pollution in these very fragile materials, which contain a large amount of partly exchangeable water. However, he observed a 40% variation of the D/H ratio and showed that some fractions of Orgueil and Mokoia contained hydrogen enriched up to 20% relative to SMOW, the upper range of deuterium enrichment (155.76 p.p.m.) for most terrestrial hydrogen components. Hence these fractions were probably traces of genuine extra-terrestrial D/H ratios. They show an approximately 10-fold enrichment relative to the primordial D/H estimate defined above. Little work was then done until, in 1975, we studied ordinary chondrites to ascertain whether this enrichment was an overall feature of telluric objects[12,13]. Our analytical procedure was much more sensitive than Boato's, allowing isotopic measurements on 0.1–20 mg samples. This makes it possible to test isotopic homogeneity on these samples, which are generally < 1 g.

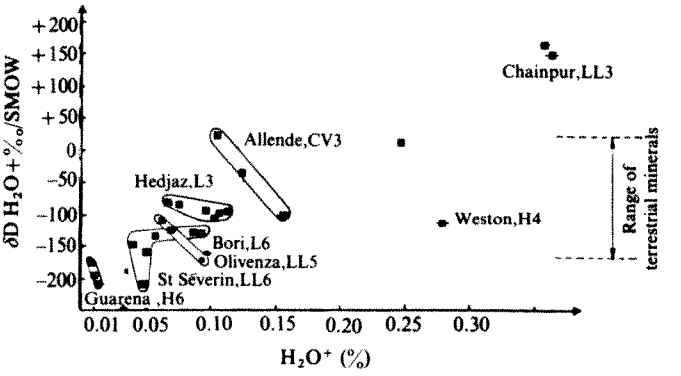


Fig. 1 D/H and hydrogen contents reported as H_2O^+ for various chondrites showing an overall positive correlation.

The results are summarised in Fig. 1, where D/H is plotted against H_2O^+ content (water extracted at $T > 200$ °C). They show a D/H variation from 124 p.p.m. for Guarena (LL6) to 220 p.p.m. for Chainpur whose conventional petrographic type is LL3 but whose matrix carbon content and chondrules' characteristics are very similar to those of carbonaceous chondrites. All these values are strongly enriched relative to the

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cosmic abundance (a factor between 6 and 11) and comparable to terrestrial values. On the other hand, we observe large variations between meteorites, and inside a single meteorite. These variations are particularly striking in the case of Chainpur. These features have been discussed[12-14] in the framework of accretion models of parent bodies and subsequent metamorphism of equilibrated chondrites. On the whole the results agree with the hypothesis of water adsorption on previously condensed grains, at temperatures varying from 180 to 230 K and total pressures of 10^{-2}–10^{-4} atmospheres, increasing with decreasing temperature, and of a corresponding increase of the D/H ratio due to a rapid increase of the fractionation coefficient $\alpha_{DH_2O-H_2}$ with decreasing temperature. Unequilibrated chondrites would completely retain these low temperature features whereas metamorphosed chondrites could have been depleted in water and deuterium by outgassing of variable quantities of deuterium enriched water (Fig. 2).

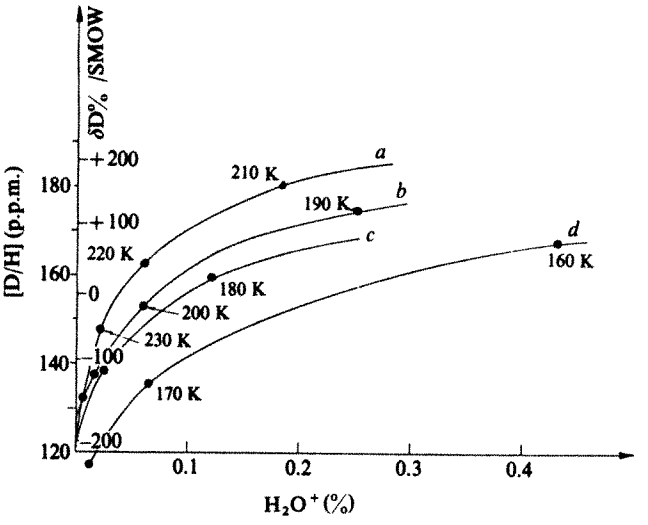


Fig. 2 Model of water adsorption on grains with fractionation H_2–H_2O_{sol} at various total pressures using data of Sill and Wilkening for total water adsorption and Richet *et al.* for $\alpha_{D\,H_2-H_2O_{sol}}$. Curves: *a* $P_T = 10^{-2}$ atm, $(D/H)_{H_2} = 25$ p.p.m.; *b*, $P_T = 10^{-3}$ atm, $(D/H)_{H_2} = 19$ p.p.m.; *c*, $P_T = 10^{-4}$ atm, $(D/H)_{H_2} = 15$ p.p.m.; *d*, $P_T = 10^{-5}$ atm, $(D/H)_{H_2} = 11$ p.p.m.

This model, while satisfactory for the whole meteorite sample, implied drastic temperature and pressure variations to explain the total range (140–218 p.p.m.) observed in a 1-g sample of Chainpur. We therefore decided to measure ^{18}O and ^{17}O abundances on the same samples, to look for possible relationships between deuterium contents and the location of these samples in a $\delta^{17}O$–$\delta^{18}O$ diagram[15]. (δD, $\delta^{17}O$ and $\delta^{18}O$ are defined as: $\delta = (R_{Sample}/R_{SMOW} - 1) \times 1{,}000$, where $R = D/H$, $^{17}O/^{16}O$, $^{18}O/^{16}O$.)

Analytical procedure

Analyses have been made on five samples (chondrules and matrix) separated by gentle crushing. The chondrules were then picked up and washed from matrix debris with acetone in an ultrasonic tank. The Chainpur matrix originally contained debris of chondrules[16] so that the 'matrix' sample is only the most enriched in matrix material in a petrological sense. Hydrogen was extracted by heating with an electrical resistance furnace.

Our previous results[12-14] were obtained by a vacuum extraction technique. For the present study, the extracted gases were carried by an helium flow through a vanadium furnace heated at 800 °C where the reduction of the hydrogen compounds takes place, and then introduced directly into the ion source of the mass spectrometer after selective diffusion of hydrogen through palladium heated at 450 °C. The gas mixture flows along a tube made of silver–palladium alloy (50 mm long and 0.08 mm thick) which is directly sealed to the line of the mass spectrometer. The flow of helium (~ 0.25 cm^3 s^{-1}) is kept small relative to the diffusion rate of hydrogen through the silver–palladium tube (~ 1 cm^3 s^{-1}). Thus the variation of the isotopic composition of hydrogen with rising temperature is continuously monitored.

Systematic calibrations are made to measure the absolute sensitivity of the whole apparatus to hydrogen and D/H ratios, using known amounts of hydrogen with a known D/H ratio. The method has been checked by the analysis of a terrestrial phengite provided by O'Neil with $\delta D = -39‰$. Three measurements of this sample gave $-40 \pm 2‰$ with a flat stepwise heating D/H release pattern between 200 and 1,200 °C.

After hydrogen extraction, oxygen was recovered by the BrF_5 technique, purified through a 5 Å molecular sieve, collected and analysed as O_2 in Micromass 602C double collecting mass spectrometer. We checked that no impurities were present, which could contribute to masses 33 or 34 (essentially NF_3 which would appear also at mass 52 (ref. 17)). Traces of CF_4 were occasionally present, which could not modify the 33 or 34 currents.

Table 1 shows the results as conventional H_2O^- (hydrogen extracted below 200 °C) and H_2O^+ (>200 °C) contents. For samples *A* and *B*, H_2O^+ has been extracted by heating between 200 and 1,150 °C at a rate of about 15 °C min^{-1}. For samples *C*, *D* and *E* temperature steps have been observed. Table 1 and Fig. 3 show that deuterium rich fraction 3 is extracted between 450 and 750 °C. The relatively high values of the D/H ratio observed in the hydrogen extracted at $T > 750$ °C may, at least partly, reflect a tailing effect of the whole extraction line, mainly in the vanadium furnace and the palladium membrane. In contrast, it is worth noting that: (1) that fraction 2 ($200 < T < 450$ °C) is not enriched in deuterium compared with fraction 1

Table 1 Oxygen and hydrogen isotope composition of Chainpur samples

Samples	H_2O (10^{-4} g per g)	D/H (p.p.m.)	$\delta^{17}O‰$ (SMOW)	$\delta^{18}O‰$ (SMOW)
A $m = 10.7$ mg				
1 chondrule				
(a) H_2O^- 20–200 °C	5.3	165		
(b) H_2O^+ 200–1200 °C	5.5	326	+3.2	+4.9
B $m = 15.2$ mg				
6 chondrules				
(a) H_2O^- 20–200 °C	9.2	153		
(b) H_2O^+ 200–1200 °C	6.0	201		
C $m = 21.1$ mg				
10 chondrules				
(a) H_2O^- 20–200 °C	4.7	157		
(b) H_2O^+ 200–450 °c	3.0	155		
450–750 °C	0.5	846		
750–1200 °C	0.7	242		
H_2O^+ mean	4.2	252	+5.6	+7.5
D $m = 9.1$ mg				
8 chondrules				
(a) H_2O^- 20–200 °C	3.9	161		
(b) H_2O^+ 200–450 °C	2.7	154		
450–750 °C	1.3	471		
750–1200 °C	0.1	199		
H_2O^+ mean	4.1	256	+4.2	+5.4
E matrix				
(a) H_2O^- 20–200 °C	24	156		
(b) H_2O^+ 200–450 °C	11	146		
450–750 °C	3.4	672		
750–1200 °C	1.8	350		
H_2O^+ mean	16.2	279	+3.5	+4.1

Oxygen data refer to whole sample after hydrogen extraction by step heating experiments.

(H_2O^-); and (2) that no correlation is observed between the isotopic ratios of fractions 2 and 3 (Fig. 3).

The values of D/H measured in fraction 3 of samples *D* and *E* are the highest ever recorded in natural samples. D/H ratios up to 300 p.p.m. have been measured[18] in a lunar rock irradiated by cosmic rays, where the high values of D/H were observed in fractions extracted at $T < 470$ °C in a back ground of normal, deuterium free, lunar hydrogen of solar-wind origin.

The $\delta^{17}O/\delta^{18}O$ ratio (Table 1 and Fig. 4) diagram[15] enables a distinction to be made between physicochemical (mass dependent) fractionations, which correspond to variations along a straight line of gradient $\frac{1}{2}$ and nuclear effects which define other correlations or possibly no correlation at all.

Chainpur results are variable, and, except for sample *A* plot well above the mean value for LL chondrites measured by Clayton *et al.*[17] defining approximately a straight line of gradient $\frac{1}{2}$ translated by +0.5‰ in $\delta^{17}O$ relative to the LL straight line. Hence Chainpur samples contain more ^{17}O than any other object in the Solar System or less pure-^{16}O component. This is related to the fact that Chainpur is the only LL3 analysed to date and work in progress suggests that even larger oxygen isotope heterogeneities exist in this meteorite and other type 3 chondrites.

Discussion

There are two main explanations for such results, one relating to physicochemical fractionations and one to nuclear effects and they will probably have to be mixed to some extent.

The driving mechanisms are: temperature variations for the physicochemical fractionation, mixing with presolar grains or heavy energetic irradiations for nuclear effects (oxygen, magnesium, neon, and so on).

Until the discovery of the $^{17}O/^{18}O$ anomalies in the meteorite Allende[16] oxygen isotope variations in meteorites had been mainly explained in terms of physicochemical fractionations. The few D/H results, since deuterium formation is attributed almost exclusively to the big bang[1,2], have also been explained by physicochemical fractionations[19] eventually associated with mixing to deuterium free solar wind[10]. The departure from physicochemical effects can be demonstrated if more than two isotopes are present (oxygen) and is much more difficult for elements such as hydrogen which have only two stable isotopes. The main indication for an isotope anomaly in these elements would be the size of the isotopic effect and an eventual correlation with other definitely nuclear effects.

Although irradiation models have recently been considered for oxygen[20–22], the variations are so large and most of them so well correlated on a $\delta^{17}O/\delta^{18}O$ diagram[15,17] that the hypothesis of presolar grains resulting from the explosion of a nearby super nova[15] and carrying pure ^{16}O, is still more convincing. Chainpur would be the least contaminated by this pure ^{16}O component. Isotopic variations among samples *C*, *D* and *E* could be attributed mainly to physicochemical fractionations whereas sample *A* would reflect a different proportion of ^{16}O component. Alternatively this departure from the straight line with gradient 1/2 could be due to ^{17}O and ^{18}O created from spallation reactions.

Temperature dependent fractionation

The step heating process reveals for hydrogen, inside a given fraction, large heterogeneities with enormous D/H ratios. To account for these ratios in the framework of water adsorption at low temperatures, we have to consider temperatures down to 110 K. In a simple black-body model this corresponds to a distance of about 3 AU, the location of the asteroid belt, but this cannot be used as a definitive argument since Wetherill[23] demonstrated that the asteroid belt is a kind of gravitational well for small objects coming from a large region of the Solar System. Measurement on interstellar clouds from the Orion nebula[25] shows an enormous D/H ratio in the HCN molecule, more or less correlated with the temperature down to 10 K, which could be obtained by equilibrium or kinetic, temperature dependent, fractionations.

Hence the observed D/H distribution in Chainpur may be the result of a very complex mixing of hydrogen compounds condensed at various temperatures in the solar nebula or even remnants of the interstellar medium. However, the fact that D/H fractionations in Orion depend on the temperature down to 10 K indirectly suggests that any change to a higher temperature would redistribute the D/H ratios. In particular in chondrules, which were strongly heated and probably fused, we should have uniform D/H ratios. This, together with the medium temperature range (450–750 °C) at which we obtain the D/H peak is against the survival of volatile molecules and in support of the creation of anomalies after the chondrules formation.

Early irradiation model

Deuterium can be created in various environments. Besides the big bang which is the most favoured model, two hypotheses directly relate to the early history of the Solar System. The first one is the effect of shock waves in supernovae envelopes[27]. The conditions postulated for the production of deuterium in such envelopes are far different from those considered for the production of the isotopically anomalous presolar grains and require energies $>10^{53}$ erg, which have not been observed. Also our deuterium enrichments have been found in the ^{17}O and ^{18}O

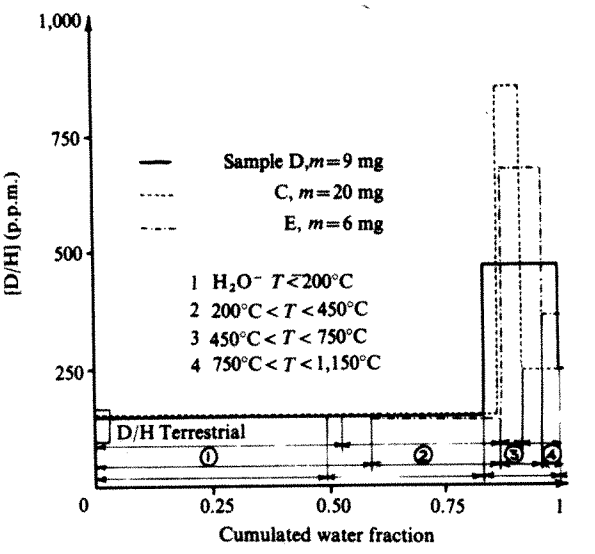


Fig. 3 D/H and water contents extracted during stepwise heating experiments of Chainpur samples, corresponding to the data of Table 1.

richest material which is exactly the opposite of what is expected of presolar grains originated from supernovae. The second involves an early irradiation within the Solar System. This hypothesis is rejected by Reeves *et al.*[2] and seriously questioned by Epstein *et al.*[26] for deuterium production, it also has been considered to explain neon, oxygen, magnesium and other isotope anomalies discovered in the 1970s. It is finally rejected by Clayton *et al.*[21] and proposed with special and somewhat *ad hoc* limitations by Lee[22]. However, our very special deuterium anomaly leads us to reconsider it.

If this model proves to be reasonable, we shall also have to consider its logical extension: not only the D/H ratio is heterogeneous in Chainpur but every chemical and petrological feature and this is due to the fact that it is an unequilibrated

chondrite. If the early irradiation affected Chainpur, it possibly also affected the parent material of larger objects such as the Earth where the deuterium was largely redistributed and homogenised. Hence this irradiation can be responsible for the production of a large proportion of the deuterium on Earth and other large objects even if the original signature has been lost by homogenisation. If energetic and cosmochemical evidence is not violated this hypothesis cannot be ruled out.

In such a model first we have to estimate which part of the deuterium measured on our experiments is the spallation product: that corresponding to the peak of very high D/H, the deuterium excess relative to the equilibrium content relative to the nebula at a given temperature, or the whole deuterium content. Given the importance of the high D/H peak, the three possible choices give results comparable within an order of magnitude. For simplicity, we have reported in Table 2 the deuterium quantities corresponding to H_2O^+ (hydrogen extracted at $T > 200$ °C).

We also have to decide what is the possible contribution of terrestrial contamination: for all our experiments on chondrites[12,13], it has been considered negligible above 200 °C.

Several spallation reactions by protons produce deuterium with reasonable yields. For a power law energy spectrum ($d\phi/dE = Ke^{-\gamma}$ with $2 < \gamma < 5$) and low energy cutoffs of <10 Mev, these reactions typically have effective cross-sections of the order of 0.1 b (ref. 21). The most efficient should be on oxygen and iron targets in the solid state and helium in the gaseous state. We cannot get such high D/H in the gas without unreasonably high fluences. In the solid, due to the high (Fe + O)/H ratio, necessary fluences are much lower. They are listed in Table 2 where they range from 10 to 10^{20} cm^{-2}. These fluences could be notably reduced (down to a factor of 10) if capture of secondary neutrons produced by spallation were also considered[3]. However, for this process to be really efficient, we need thermalised neutron fluences up to 10^{20} cm^{-2} which should show up on the isotopic composition of elements such as gadolinium, which should be looked for in Chainpur. Until then we shall assume that the necessary proton fluence is 10^{19}–10^{20} cm^{-2} which is comparable to that considered by Clayton *et al.*[21] to produce the ^{26}Mg anomaly in Allende but much less than the fluence needed to produce the maximum oxygen anomaly (10^{23}–10^{25} cm^{-2} according to the scenario[21,22]).

Energy limitations

The next step is to decide what kind of system and what global mass was irradiated by such an effect. If the spallation deuterium is restricted to the peak and not found in other objects, this means that the irradiation is restricted to the surface of tiny objects like Chainpur parent body and the energy problem will not be too serious. The isotopic and chemical difficulties are reviewed later. However, this is an *ad hoc* scenario and this proton fluence is more likely to come from the main source of energy of the system which is the young Sun. Then all the matter exposed between the Sun and the location of Chainpur condensation (and possibly further) has to be considered. The period which we must consider is that during which solids condensate, planetary bodies accrete and residual nebula is swept away, and it might be very short (10^7–10^8 yr). The period of T Tauri radiation itself might be much shorter (10^6 yr)[29]. Possible scenarios, such as nuclear and energy needs, strongly depend on when precisely the T Tauri phase occurs in the longer period: if a planet is already formed then the effects of the radiation will be only surficial and vanish eventually by mixing. This is particularly important for the jovian planets. If the nebula has already been swept away, then the stopping distance is much larger, the mass involved and the energy needed considerably reduced. The careful examination of deuterium contents may then set the average accretion time of planets relative to the T Tauri phase and to each other.

Practically, if we want to apply a 10^{20} cm^{-2} fluence to the jovian planets or to a nebula of 10^{-5}–10^{-3} atmospheres total pressure extending over ~1 AU, we have to consider a total mass of 10^{-3}–$2 \times 10^{-1}\ M_\odot$, which leads to totally unreasonable energy dissipation.

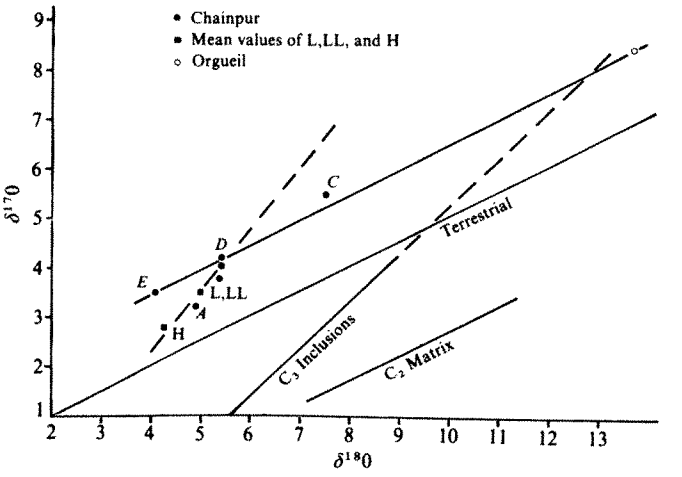


Fig. 4 $\delta^{17}O/\delta^{18}O$ plot of the isotopic composition of Chainpur samples, corresponding to the data of Table 1. ■, data from ref. 21.

In contrast, the hypothesis which suggests that the deuterium content of the terrestrial planets could largely be due to spallation is reasonable from an energetical point of view. The global D/H ratios in meteorites and the Earth vary from 140 to 220 p.p.m. and are thus very similar. The Earth probably contains most of the total deuterium estimated at 1.5×10^{43}–1×10^{44} atoms. This number cannot be more than doubled if we take into account all the terrestrial planets. If this whole mass was irradiated in its dust form to produce most of its deuterium, we can calculate the total energy needed, mostly lost by ionisation. The total mass corresponds roughly to 5×10^{50} atoms. Taking a mean ionisation cross-section of 10^{-26} erg cm^{-2} as an upper limit and making calculations similar to Lee's[22], we find a total energy of 5×10^{44} erg (of which 5×10^{39} erg only are dissipated by the formation of deuterium). This is roughly 10^{-4} of the total gravitational energy of the Sun. The only remaining problem is the question of the stopping distance; which strongly depends on the particle energy. For example, the residual trajectory is 5–15 10^{-2} g cm^{-2} for a 10 MeV proton and 55–130 g cm^{-2} for a 500 MeV proton in hydrogen and oxygen respectively[30,31]. For a 500 MeV proton, the energy dissipated by nuclear reactions would increase to 2.5×10^{41} erg but the total energy loss would eventually decrease because the stopping power is less. The total flux of such protons ($\phi \times t = 10^{20}$ cm^{-2}) across a sphere of 1.5 AU radius would amount to 2×10^{44} erg, roughly one-third of the total energy need. Whether this intermediate range of energy, postulated by

Table 2 Total deuterium contents (atoms per g), corresponding to H_2O^+ (extracted above 200 °C) and corresponding fluences needed for protons of $E > 10$ MV

Sample	D in H_2O^+ (atoms per g)	$I \times t$ (protons cm^2 s^{-1})
A	12×10^{15}	3.3×10^{19}
B	8.1×10^{15}	2.2×10^{19}
C	7.1×10^{15}	2.0×10^{19}
D	7.0×10^{15}	1.9×10^{19}
E	30.2×10^{15}	8.4×10^{19}

Fowler *et al.*[2] is realistic is debatable but it is energetically reasonable. It provides much longer travel distances, which would allow the protons to penetrate deeply (~40 cm) into planetesimals, and not only dust, and also to travel 1 AU into a 3×10 g cm^{-3} (3×10^{-8} atm) nebula.

Cosmochemical constraints

The total amount of deuterium formed is only 3×10^{-8} of the estimated protosolar deuterium. Hence the global deuterium cosmology is not strongly modified inasmuch as only 7–30% of the matter is supposedly reprocessed in stellar systems. But the early irradiation can have very strong effects on other elements in the irradiated materials, especially on Li, Be, B, rare gases, magnesium (^{26}Mg) and also on rarer nuclei such as ^{50}V, ^{92}Nb, ^{138}La, ^{180}Ta (refs 21, 22). As most of the latter nuclei still have to be investigated, we only consider the problem of light elements and mention the rare gases limitations.

We stress that our deuterium anomaly does not apply to the same type of material as Allende's inclusions (as is reflected by the mineralogy and also by the ^{17}O ^{18}O results), but the postulated fluences are similar to that proposed by Clayton *et al.*[21]. They would produce rather significant amounts of Li, Be and B by spallation on oxygen, carbon and nitrogen[32] and also large amounts of rare gases among which the already discussed ^{22}Ne, and ^{36}Ar and ^{38}Ar from ^{39}K and ^{41}K respectively[21]. They could also produce ^{17}O and ^{18}O by spallation on the Si group, with rather small cross-sections (~10 mb), but, because of the large abundance of the Si group about 10 times more efficient than the destruction reactions.

These spallations (mainly on Si and Mg), could produce 10^{15}–10^{16} atoms g^{-1} of ^{17}O and ^{18}O which corresponds to variations in δ^{17}O of 0.15 to 1.5‰ and in δ^{18}O of 0.03 to 0.3‰. The ^{17}O variations approximately match the total offset relative to a straight line with a gradient of $\frac{1}{2}$ whereas the ^{18}O variations are much smaller than the observed values. This can reflect the result of two different processes: (1) the proton irradiation, acting mainly on ^{17}O and (2) the effect of mass dependent fractionations, occurring, for example, during the formation of the chondrules, which would bring the isotopic composition of the condensed phases from δ^{18}O ~ 4‰ closer to the nebular composition (+13 to +14‰ after the intercept between Chainpur line and Allende's inclusions line).

Consider then the Li, Be and B: using cross-sections of 10–20 mb (ref. 29), we calculate for our maximum influence of 10^{20} cm^{-2} a production of about 0.3 p.p.m. of Li and about twice as much B. The production of Be should approach 0.01 p.p.m. If we compare this to the observed concentrations[33–35] which range from 1 to 3 p.p.m. for Li and B, we have to account for a 10–60% increase, which from an elemental point of view is perfectly acceptable.

The most severe limitations come from the isotopic composition as there are large differences between meteoritic and spallation-produced ^{7}Li/^{6}Li. Observed meteoritic ^{7}Li/^{6}Li are up to now equal within a few per cent to 12.5 including Chainpur[33] whereas spallation Li has ^{7}Li/^{6}Li ranging from ~3 to 7 depending on the energy range. The data of Chainpur show that we should expect a decrease of ^{7}Li/^{6}Li from 12.7 to 12 or 11.4, which should be perfectly measurable.

Now our model attempts to explain essentially all the observed D/H ratios in terrestrial objects and for them we need comparable fluences. For the Sun and stars we have only a lower limit for ^{7}Li/^{6}Li and the only accurate numbers are for terrestrial objects, so the observed homogeneity may be the result of mixing in almost constant proportions of lithium from three sources: ^{7}Li from the big bang, ^{7}Li and ^{6}Li from galactic cosmic radiation, producing only a few per cent variation. Note that this remarkable homogeneity remains to be satisfactorily explained even if we remove the third component.

The postulated fluences produce too much ^{22}Ne in the solid as discussed by Clayton *et al.* For Chainpur (0.1% K) the ^{36}Ar production should be $0.5\text{–}5\times10^{-6}$ cm^3 STP g^{-1}, which satisfactorily matches the results obtained by Podosek[36] (0.85 to $>1.18\ 10^{-6}$ cm^3 STP g^{-1}). The ^{36}Ar/^{38}Ar spallation ratio should vary between 15 and 70 according to the energy spectrum[21]. The ratio observed by Podosek is 3.5–4.0 in an irradiated sample. If ^{38}Ar is corrected for ^{38}Ar produced from ^{37}Cl (^{37}Cl(n, γ, β)^{38}Ar) as for Karoonda[36,37], the ratio is 10–14, very different from the solar value. However, other results on Chainpur[38] do not confirm this result. Also, the mechanisms by which the Earth can rebuild a solar ^{36}Ar/^{38}Ar after the early irradiation remain to be imagined precisely.

Conclusion

In the meteorite Chainpur we have found very large anomalies in deuterium contents associated with small enrichments in ^{17}O and larger variations along a straight line with gradient 1/2 in a δ^{17}O/δ^{18}O diagram. Deuterium results can be explained by a temperature dependent model of fractionation in a wide range of low temperatures, but an early irradiation during the T Tauri phase is presently favoured, which could explain most of the hydrogen and oxygen isotope features. The extension to C_1 chondrites is very difficult because the deuterium amounts are 20 times larger.

We thank P. Pellas for samples. J. Audouze for his kindness, and J.-F. Minster, C.-J. Allegre and E. Roth for helpful comments.

Note added in proof: Since this report was written Clayton and Mayeda found very similar results to the Chainpur samples on a LL3 chondrite recovered from Antarctica (ANS) (see ref. 40).

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Nebular condensation of Ga, Ge and Sb and the chemical classification of iron meteorites

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Key parameters for classifying iron meteorites into genetic groups are concentrations of Ga and Ge. Their taxonomic value results from the combination of very wide concentration ranges in iron meteorites as a whole, with very narrow concentration ranges in most individual groups. The very wide intergroup ranges result from solar nebular condensation in widely varying conditions. Recent calculations show that the nebular condensation temperature of Ge is lower than that of any other siderophile, and that Sb and Ga are the next most volatile siderophiles. The narrow intragroup ranges of Ga and Ge reflect minimal fractionation during the crystallisation of cores, larger intragroup ranges for Sb result from a solid/liquid distribution ratio near 0.4.

Only during the past 20 yr have meteorite researchers developed a genetic classification for iron meteorites[1-5]. This system, often called the chemical classification, is based on a combination of metallic structure and the concentration in the metal of several siderophile (metal-preferring) elements, most especially Ga and Ge[6-8]. The Ga–Ni and Ge–Ni fields occupied by the 13 groups of iron meteorites are shown in Fig. 1. Although the total concentration ranges among all meteorites are 1,600 and 400,000 for Ga and Ge, respectively, only the two groups IAB and IIICD have maximum/minimum ranges >2. There is evidence which indicates that the groups having wider ranges were never fully molten; their large Ga and Ge ranges may have been established during nebular fractionation[9,10] or partial melting processes[11]. Most evidence favours the view that the remaining 11 groups formed in planetary cores that were once fully molten[12,13].

The explanation for the Ga–Ge quantisation has been sought for the past two decades. Anders[14,15] and Scott[12] attributed the large concentration ranges to nebular volatility, but were not able to explain why other siderophilic elements that appeared to be equally or more volatile did not exhibit similar distributions. Wai *et al.*[16] examined the possibility that the low Ge contents could have resulted from preferred partitioning into silicate (mantle) materials; their experiments showed that Ge was highly siderophile in temperature and redox conditions appropriate to core formation.

Non-nebular fractionations have produced large intragroup concentration range of some elements (such as, Ir, Os and Re ranges of a factor 5,000 are observed in group IIAB), requiring extreme care when estimating group means. To calculate intergroup ranges we have reviewed published data[17,18] and used treatments involving fractional crystallisation equations or weighting to achieve precise mean compositions[19], then calculated mean abundances relative to Ni. The six highest maximum/minimum abundance ranges are 15,400, 890, 138, 55, 36 and 11.4 for the elements Ge, Ga, Sb, As, Cu, and Au, respectively. We have previously calculated 50% condensation temperatures for several moderately volatile elements and showed that there was an approximately monotonic decrease in the ordinary-chondrite/CI-chondrite abundance ratio with decreasing nebular condensation temperature[20]. As expected, the maximum/minimum abundance ranges in iron meteorites are also negatively correlated with our previously published condensation temperatures, but an important exception is that the Ga range is higher than that of Sb despite the fact that our previously calculated Ga condensation temperature was 150 K higher than that for Sb.

Nebular equilibria

The correctness of equilibrium condensation calculations depends largely on the quality and completeness of available thermodynamic data of the relevant gaseous and condensed forms of the element under consideration. For example, if lack of data results in exclusion of a stable gaseous form of an element from the calculation, the calculated condensation temperature will be too high. If a minor or trace element condenses in solid solution with a major mineral phase, calculation of a correct condensation temperature requires inclusion of an appropriate activity coefficient (γ) of the element in the host phase. In most previously reported condensation temperatures γ was taken equal to unity, that is, ideal solution behaviour was assumed. In our published calculations[20] we estimated γ values that were assumed to be independent of temperature.

In fact, the activity coefficient depends both on temperature and composition. The relationship of γ to the temperature T, the partial molar heat of solution $\Delta\bar{H}_s$ and the excess partial molar entropy of mixing $\Delta\bar{S}^{xs}$ is given in standard thermodynamics texts[21]:

$$\ln\gamma = \frac{\Delta\bar{H}_s}{RT} - \frac{\Delta\bar{S}^{xs}}{R} \quad (1)$$

where R is the gas constant. Based on available data, we have estimated the activity coefficients of the six most volatile siderophilic trace elements in Fe–Ni according to equation (1). The partial molar quantities for each trace element given in Table 1 are for a dilute solid solution with the mole fraction (X) of the element approaching zero; the Fe:Ni mole ratio is assumed to be 12. The quality and suitability of the available thermodynamic data vary, as described below.

The activity coefficient of Au in Ni (γAu[Ni]) at 1,150 K approaches 10 as X_{Au} approaches zero[22]. At 1,123 K γAu[Fe] data are available only for X_{Au} from 1.0 to 0.45; the trend follows closely that of γAu[Ni]. Therefore, the $\Delta\bar{H}_s$ and $\Delta\bar{S}^{xs}$ for Au in Fe–Ni were assumed to be identical with those for the Au–Ni system. Activity coefficients of Cu in solid Ni or Fe are not available, but γCu[Ni] and γCu[Fe] at $X_{Cu} = 0$ have been reported for liquid systems at 1,823 K (ref. 22). Solid phase γ

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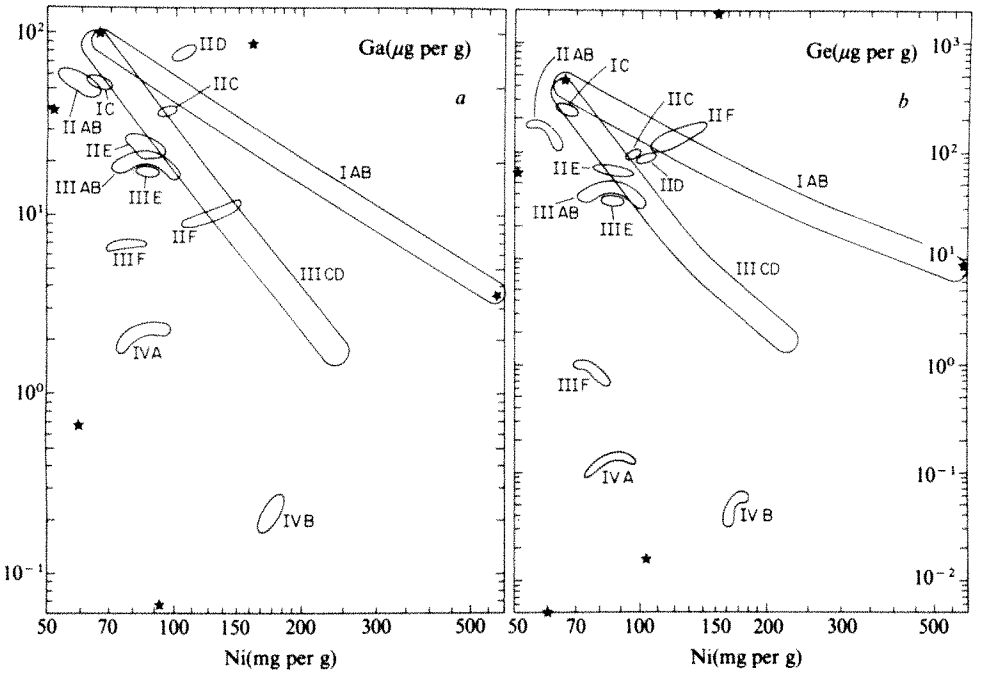


Fig. 1 log–log Ga–Ni (*a*) and Ge–Ni (*b*) diagrams; note differences in the logarithmic scales. The large concentration ranges in the non-magmatic groups IAB and IIICD reflect the fact that they were not subjected to the homogenisation associated with core formation. The 11 groups that formed on cores show quantised Ga and Ge ranges (factors ≤1.7) and minimal overlap. Stars show the locations of the iron having maximum and minimum concentrations of these three elements; two are members of group IAB and the remainder ungrouped.

values were calculated based on iron meteoritic evidence that the equilibrium partition coefficient of Cu between liquid and solid Fe–Ni is close to unity, that is, the γ value of Cu in solid Fe–Ni should be close to that for liquid Fe–Ni (ref. 19). From these data we estimated the Cu[Fe–Ni] partial molar quantities.

We estimated partial molar quantities for Ge in Fe–Ni from infinite dilution γGe[Fe] and γGe[Ni] values at 1,800 K (refs 23, 24), and from a solid/liquid distribution coefficient of 0.89 observed in low-Ni group IIIAB iron meteorites[19]. Extrapolation below $X_{As} = 0.11$ yielded γAs[Fe] = 0.5 at infinite dilution at 1,573 K (ref. 25). We estimated the partial molar quantities for As in solid Fe–Ni from these data and from the As solid/liquid distribution coefficient of ~0.41 inferred for group IIIAB (ref. 19). Partial molar quantities for these four siderophiles are based on high-temperature experimental data from dilute binary solutions and from meteoritic measurements. A major source of uncertainty in this application is the assumption that $\Delta\bar{H}_s$ is temperature independent over a wide temperature range. An error of 1–2 kcal mol^{-1} in the estimated $\Delta\bar{H}$ values would change the 50% condensation temperatures by 10–30°. Our estimated uncertainties are of this order.

The enthalpies of formation in solid Ga–Fe and Ga–Ni systems have been reported to exhibit large negative deviations with maximum values of −8.5 kcal mol^{-1} (ref. 26) and −11.5 kcal mol^{-1} (ref. 27), respectively. Hultgren *et al.*[22] give a γGa[Ni] at 1,223 K of 3.3×10^{-3} at $X_{Ga} = 0.37$. Kelly and Larimer[11] suggested a lower γGa[Fe–Ni] of 3×10^{-3} which we find more in keeping with the lower $\Delta\bar{H}_s$ data for Ga–Fe, and our partial molar quantities were chosen accordingly.

Like its congener As, Sb forms stable compounds with Fe and Ni (ref. 21). The integral heat of solution and excess entropy of mixing of Sb in solid Fe at 800 K and $X_{Sb} = 0.45$ are −2.3 kcal mol^{-1} and −2 cal deg^{-1} mol^{-1}, respectively[22] and γ is 0.23 at $X_{Sb} = 0.3$ at 893 K (ref. 28). However, data for dilute Sb–Fe or Sb–Ni solid solutions are not available. Takahashi *et al.*[29] used $\gamma = 1 \times 10^{-3}$. This γ value at a typical Sb condensation temperature of ~900 K corresponds to a $\Delta\bar{H}_s$ of −14 kcal mol^{-1} if $\Delta\bar{S}^{xs}$ is −2 cal deg^{-1} mol^{-1}. This $\Delta\bar{H}_s$ is implausibly more negative than that estimated for As in Fe–Ni, thus we chose a smaller $\Delta\bar{H}_s$ value. The partial molar quantities of Sb given in Table 1 are more in keeping with the expectation that, given their closely similar electronic configurations, γ for Sb (radius ~1.6 Å) should be somewhat larger than that for As (radius ~1.5 Å) since As fits better into Fe–Ni lattice sites (mean radius ~1.28 Å)[30].

Because of the paucity of data, the relative uncertainties in the estimated partial molar quantities for Ga and Sb are ~±30%. The errors in our calculated condensation temperatures for Ga and Sb caused by uncertainty in their activity coefficients are probably twice as large as those for Au, Cu, As and Ge. Despite the uncertainties in their activity coefficients we believe our calculated condensation temperatures for these elements are the most accurate allowed by currently available data.

We need to revise our previous results[20] regarding the distribution of Ga, Ge, and Sb among nebular gaseous species. Recently published data on GaOH(g)[31] show it to be an important species, and Sears[32] showed that GaCl(g) is also important. The nebular condensation of Cl is poorly understood, but it is more depleted than Ga in ordinary chondrites[33,34], suggesting that its condensation temperature is lower. Although the low abundance of Br limits its importance, GaBr(g) is also a stable nebular species[35]. Based on the assumption that Cl condensed after Ga, we evaluate the relative abundances of Ga(g), GaOH(g), GaCl(g) and GaBr(g) from the

Table 1 Equilibrium nebular 50% condensation temperatures of some moderately volatile siderophilic elements

Element	Gaseous form	Condensed form (host)	Solution data $\Delta\bar{H}_s$	Solution data $\Delta\bar{S}^{xs}$	Condensation temperatures (K) 10^{-4} atm	Condensation temperatures (K) 10^{-6} atm
Au	Au(g)	Au (Fe–Ni)	6.0	1.5	1,225	1,074
As	As(g)	As (Fe–Ni)	−9.5	−2.5	1,157	1,012
Cu	Cu(g)	Cu (Fe–Ni)	11.0	2.0	1,037	910
Sb	SbS(g)	Sb (Fe–Ni)	−7.0	−2.0	912	767
Ga	GaCl(g)	Ga (Fe–Ni)	−9.0	−2.5	918	738
Ge	GeS(g)	Ge (Fe–Ni)	−16.0	−3.6	825	702

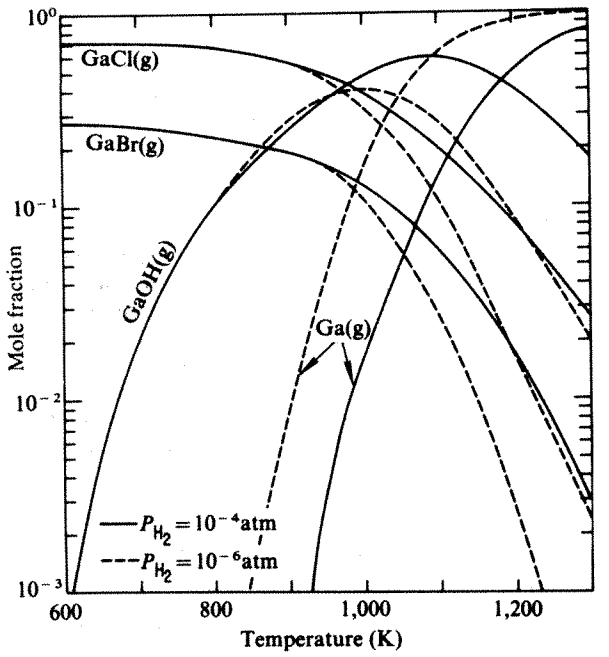


Fig. 2 Distribution of Ga among gaseous species in the solar nebula at pressures (P_{H_2}) of 10^{-4} and 10^{-6} atm. At high temperatures Ga is dominant, but with decreasing temperature first GaOH then GaCl become the most abundant gaseous forms.

following equilibrium processes:

$$GaOH(g) + 1/2H_2(g) = Ga(g) + H_2O(g)$$

$$GaOH(g) + HCl(g) = GaCl(g) + H_2O(g)$$

$$GaCl(g) + HBr(g) = GaBr(g) + HCl(g)$$

The solar abundance of Cl (4,350, Si = 10^6) using in this calculation was taken from the Dreibus *et al.*[34] data or CI chondrites; the Br abundance (9.7, Si = 10^6) is based on unpublished data of B. Spettel, H. Wänke and G. W. Kallemeyn. The remaining abundances are from Cameron[36]. The relative abundances of these four Ga species in the temperature range 600–1,200 K at nebular pressures (P_{H_2}) of 10^{-4} and 10^{-6} atm are shown in Fig. 2. The abundances of GaO(g) and $Ga_2O(g)$ are insignificant in this temperature range. The condensation of Ga was calculated from:

$$GaCl(g) + 1/2H_2(g) = Ga(s, \text{in Fe–Ni}) + HCl(g)$$

with the mole fraction of GaCl(g) corrected using the calculated distribution curve shown in Fig. 2.

The abundance of GeS(s) was underestimated in our previous study[20]; we concur with Sears[32] that it is the most abundant gaseous species of Ge. The condensation of Ge was calculated from available thermodynamic data[37,38] by the following equilibrium process:

$$GeS(g) + H_2(g) = Ge(s, \text{in Fe–Ni}) + H_2S(g)$$

The partial pressures of GeS(g) used in our calculation were corrected for the minor amounts of GeSe(g) and GeTe(g) in the nebula[39].

Our[20] Sb condensation calculations are in need of revision to allow for the fact that SbS(g) is more abundant than Sb(g) at temperatures below 1,150 K. The condensation of Sb was recalculated from free-energy functions[39] and $\Delta\bar{H}_f^0$ (298 K)[40] data:

$$SbS(g) + H_2(g) = Sb(s, \text{in Fe–Ni}) + H_2S(g)$$

The condensation processes for the other three siderophiles Au, Cu, and As as discussed previously[20] seem to be correct, but condensation temperatures have been recalculated to include the temperature dependent γ values.

The revised 50% condensation temperatures for Au, As, Cu, Sb, Ga, and Ge at nebular pressures of 10^{-4} and 10^{-6} atm are given in Table 1. As in our previous study, Ge is the most volatile siderophile. The revised Ga 50% condensation temperature is now similar to, or slightly lower than, that of Sb; thus these two elements are the next most volatile elements siderophilic at temperature and redox conditions appropriate to core formation. Our condensation calculations support the idea that the extremely large range in mean group abundances for these elements are a direct reflection of their high volatility in the nebula. In groups having low abundances they seem to have been lost to a large degree during condensation and accretion in the nebula, although we cannot exclude the possibility that some volatile loss occurred in the parent body during the process of melting and core formation.

Fractionation during core solidification

One question remains. If the volatility of Sb is nearly as great as that of Ga, why is it not 'quantised' to nearly the same degree? The answer is to be found in Fig. 3, which shows Ga–Ni, Ge–Ni and Sb–Ni distributions in IIIAB, the largest iron meteorite group. Whereas the Ga and Ge maximum/minimum ratios are 1.3 and 1.6, respectively, that for Sb is about 5. As discussed by Scott[12] and Wasson[13], there is little doubt that the element–Ni patterns in IIIAB formed by fractional crystallisation of a molten core. During fractional crystallisation elements having solid/liquid distribution coefficients near unity undergo minor fractionations, whereas those having more extreme distribution coefficients undergo large fractionations. This statement

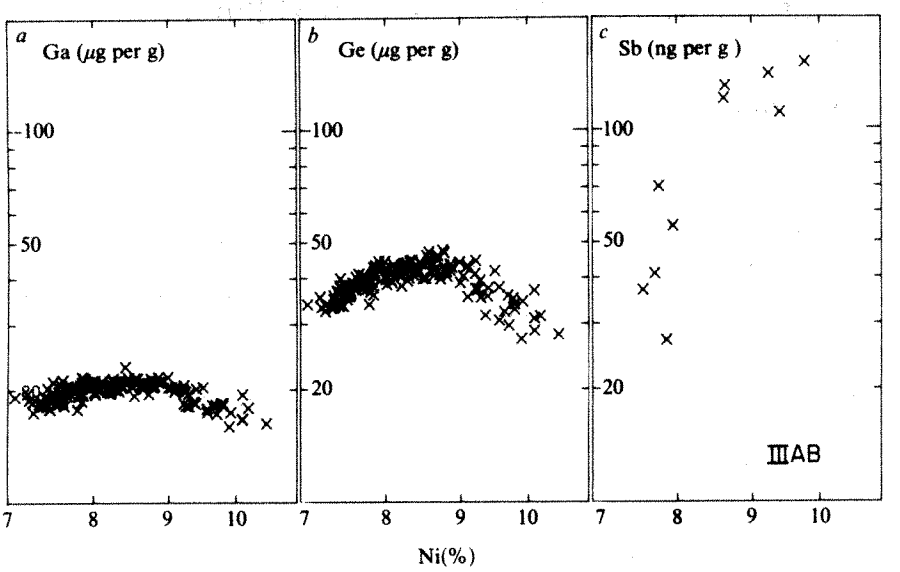


Fig. 3 A detailed comparison of the Ga–Ni (*a*), Ge–Ni (*b*) and Sb–Ni (*c*) distributions in group IIIAB. The small range of Ga and Ge produced during fractional crystallisation results from solid/liquid distribution coefficient near unity (slightly below 1 at low Ni, above 1 at high Ni concentrations). The larger Sb range (factor of 5) reflects a distribution coefficient distinctly different from unity (the estimated value is 0.44).

remains correct even if partial melting or fractional melting were responsible for the observed fractionations.

The distribution coefficient of Ni seems to have remained constant at ~0.90 during the fractional crystallisation of most iron meteorite cores[12,22]. Given this value one can calculate the distribution coefficients of elements linearly related to Ni on log–log plots. For example, the negative linear correlation of Ir and Ni in IIIAB leads to a distribution coefficient of 4.1 and a remarkable maximum/minimum ratio of 2,000. The positive linear correlation between Sb and Ni (Fig. 3) leads to a distribution coefficient of 0.44 and a maximum/minimum ratio of about 5.

In contrast, Ga and Ge are not linearly related to Ni. At low Ni concentrations the slope is positive indicating a distribution coefficient below 1 and at high concentrations it is negative indicating a distribution coefficient above 1. The iron meteorite groups originating in cores show positive Ga– and Ge–Ni correlations at low Ni concentrations, and groups IIAB and IVA also show the shift to negative slope at high Ni concentrations. In all 11 cases the maximum/minimum ratios are <2, indicating distribution coefficients near 1. The core-origin group other than IIIAB in which the Sb–Ni distribution is reasonably well defined is IIAB: its Sb range is a factor of 3. Fragmentary data for other groups indicate that ranges of 3–5 are typical for Sb (ref. 41).

Conclusion

The quantisation of Ga and Ge that is so important for the classification of iron meteorites resulted from the fortuitous combination of high nebular volatility, homogenisation during core formation, and low degrees of fractionation during fractional crystallisation of the solidifying cores.

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Thionine coated electrode for photogalvanic cells

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The successful operation of a photogalvanic cell for solar energy conversion requires that the illuminated electrode should discriminate between the two redox couples in solution. In the iron–thionine system the electrode must oxidise photogenerated leucothionine but not reduce the photogenerated Fe(III). Modified electrodes with coatings of thionine of up to 20 monolayers can be prepared on Pt and SnO_2. Electrode kinetic results for the Fe (III) and thionine systems show that this modified electrode is suitable for the iron–thionine photogalvanic cell.

THE criteria required for a successful photogalvanic cell for solar energy conversion has been discussed elsewhere[1]. The reaction scheme of the iron–thionine system for such a cell works as follows:

$$\mathrm{Th} \xrightarrow{h\nu} \mathrm{Th}^*$$

$$\mathrm{Th}^* + \mathrm{Fe(II)} \xrightarrow[\mathrm{H}^+]{} \mathrm{S}^{\cdot} + \mathrm{Fe(III)}$$

$$\mathrm{S}^{\cdot} + \mathrm{S}^{\cdot} \rightarrow \mathrm{Th} + \mathrm{L}$$

Illuminated electrode

$$\mathrm{L} \rightarrow \mathrm{Th} + 2\mathrm{e} + 3\mathrm{H}^+$$

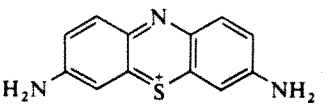
Dark electrode

$$2\mathrm{Fe(III)} + 2\mathrm{e} \rightarrow 2\mathrm{Fe(II)}$$

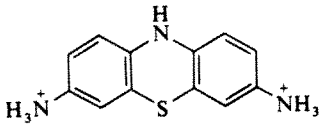
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where Th is



L is



and S˙ is the semithionine radical.

To obtain power from the cell the illuminated electrode must be able to discriminate between the photogenerated leucothionine (L) and Fe(III)[1,5]. If the electrode does not discriminate, then addition of the electrode reactions in the reaction scheme shows that the electrode merely catalyses the back reaction of photogenerated products into the original reactants:

$$L + 2Fe(III) \rightarrow Th + 2Fe(II)$$

The illuminated electrode must remove one of the photogenerated products, in this case L, and force the other, Fe(III), to diffuse across the cell and react on the dark electrode. Note that for an efficient photogalvanic cell the electrode kinetics of the two electrodes must be different[5]. If the Fe(III) reaction is blocked on the dark electrode, as well as the illuminated electrode, then the cell operates only as a concentration cell and produces insufficient voltage of $\sim RT/F$ (ref. 6). We describe here how to modify the illuminated electrode by coating it with thionine itself. The modified electrode then discriminates between the photogenerated L and Fe(III). A photogalvanic cell with a modified and an unmodified SnO_2 electrode will then possess the necessary differential electrode kinetics[7]. The properties of the modified thionine electrode are also interesting in that the electrode discriminates between the organic and inorganic couples studied. The reason for this discrimination will be discussed.

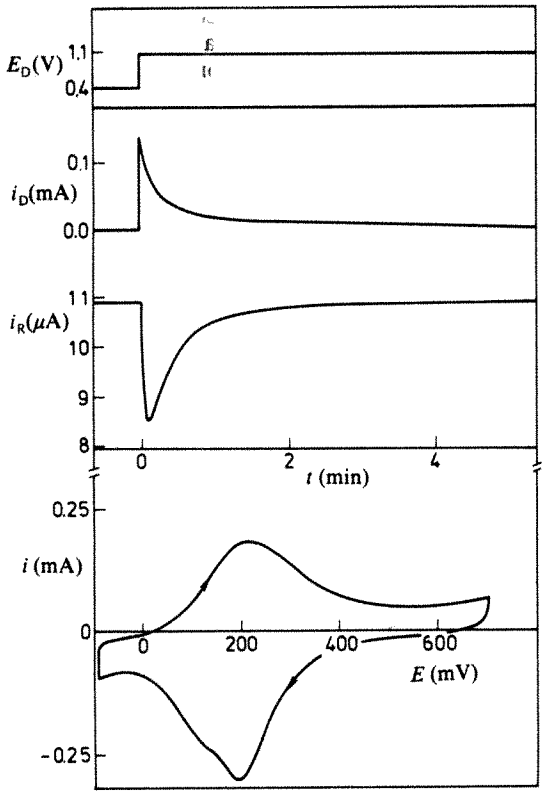


Fig. 1 Ring disk study of coating process and cyclic voltammogram of resulting thionine coated Pt electrode in 0.05 M H_2SO_4 containing no thionine. E_D is the disk potential, i_D the disk current and i_R the ring current.

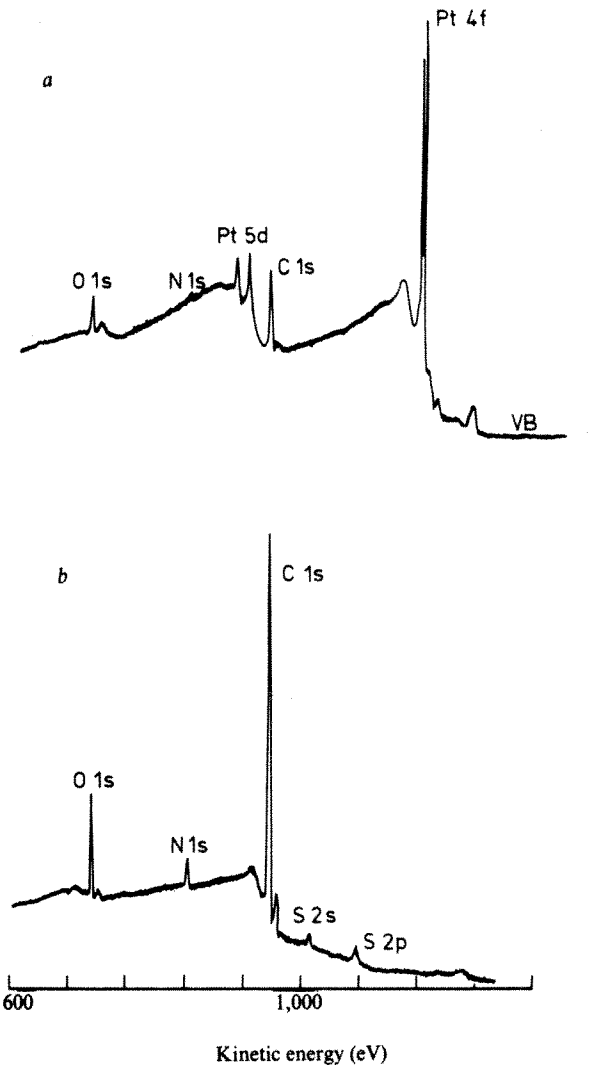


Fig. 2 Typical XPE spectra. *a*, Pt electrode that has been exposed to thionine solution at 0.4 V. The principal feature is the Pt 4f signal. *b*, Pt electrode coated at 1.1 V. The Pt 4f signal is suppressed; C, N and S corresponding to thionine are found.

Experimental methods

All the electrochemical experiments were carried out at 25 °C and all potentials reported with respect to the saturated calomel electrode. Rotating disk and ring-disk studies were performed using an Oxford Electrodes motor controller, rotating assembly and control electronics. Antimony doped SnO_2 electrodes were prepared by spraying a solution of 10 cm^3 $SnCl_4$ and 0.6 cm^3 $SbCl_5$ added to 20 cm^3 ethyl acetate for 3 s on to the end of a quartz rod heated to 1,000 °C and rotating at 5 Hz. Spectra were recorded on a Unicam SP 600 recording spectrophotometer. XPE spectra were measured on an AEI ES 200 B photoelectron spectrometer using MgKα exciting radiation; the base pressure was $\sim 10^{-9}$ torr. The conductance of the thionine coating was measured in the d.c. mode after vapour deposition of a Au contact.

Results and discussion

Preparation and properties of modified electrodes: both Pt and SnO_2 electrodes can be coated with a stable layer of thionine by holding the electrode at 1.1–1.5 V for several minutes in a solution containing thionine. Figure 1 shows a ring–disk experiment which monitors the consumption of thionine during the coating process. The thionine is coated on to the disk electrode and this process is monitored by the downstream ring electrode. From the decrease in the transport limited current for thionine at the ring electrode we can calculate from $N^{-1} \int \Delta i_R \, dt$

the amount of thionine consumed on the disk where N is the collection efficiency[8,9]. When the modified electrode is removed from the thionine solution, washed and placed in background electrolyte (such as 50 mM H_2SO_4) containing no thionine, cyclic voltamograms as shown in Fig. 1 are obtained. The peak height of these voltamograms varies linearly with sweep rate as expected for surface bound species. From the charge passed per cycle we can also calculate the amount of thionine in the layer. The coverages found from cyclic voltammetry agree with those from the ring–disk experiment but are usually about 20% lower. Coatings of up to 20 monolayers can be obtained and these coatings are stable in background electrolyte for at least several months. XPES studies, see Fig. 2, of a coated electrode show identical peaks to those of thionine and the Pt 4f peak is completely absent[10]. The cyclic voltamograms before and after the XPES experiment show that exposure to high vacuum for several hours only reduces the coverage by about 10%.

Compared with thionine in solution, the visible spectrum of a coated SnO_2 electrode on a quartz plate shows a broadened peak at much the same wavelength ($\lambda_{max}/nm \simeq 600$) (ref. 10). At reducing potentials this peak disappears. As shown in Fig. 3 the proportion of thionine, f, and leucothionine in the layer obeys the Nernst equation:

$$\frac{RT}{nF}\ln\frac{f}{1-f} = E - E_c' \qquad (1)$$

We find $n = 2$ and $E_c'/mV = 180$. This value is similar to that of thionine in our solution ($E'/mV = 208$). The resistance of 1 cm^2 of a layer coated at 1.1 V containing 6 nmol cm^{-2} was found to be 60 Ω. From these results we conclude: first, the layer consists of thionine or leucothionine depending on the electrode potential; second, the layer is remarkably stable and coherent, it cannot be washed off and the disappearance of the Pt signal in the XPE spectrum is particularly noteworthy; third, for current densities <150 μA cm^{-2} the voltage drop across the layer is <10 mV and can be neglected.

Thionine: We now examine the electrode kinetics of various redox couples on the thionine coated electrode starting with thionine. Figure 4 shows typical current–voltage curves for the reduction of thionine on a clean and a thionine coated Pt electrode. On the clean electrode the system is reversible. On the coated electrode the system is nearly reversible. Analysis of the current–voltage curves shows that the electrochemical rate constant is about 10 cm ks^{-1} for an electrode coated for 15 min at 1.1 V. Longer coating times or higher coating voltages result

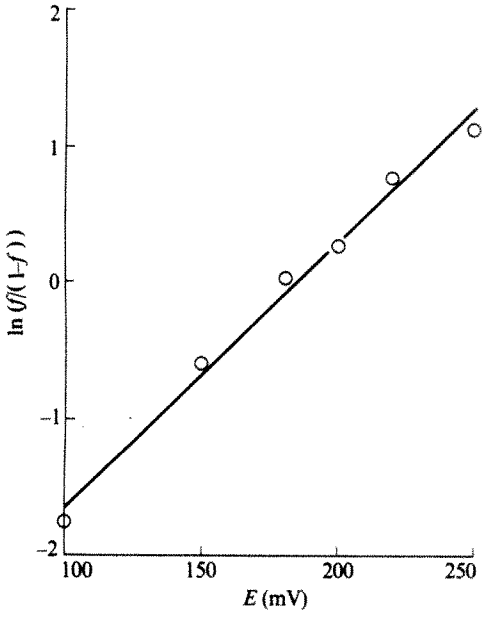


Fig. 3 Plot of Nernst equation (1) describing the bleaching of the thionine layer.

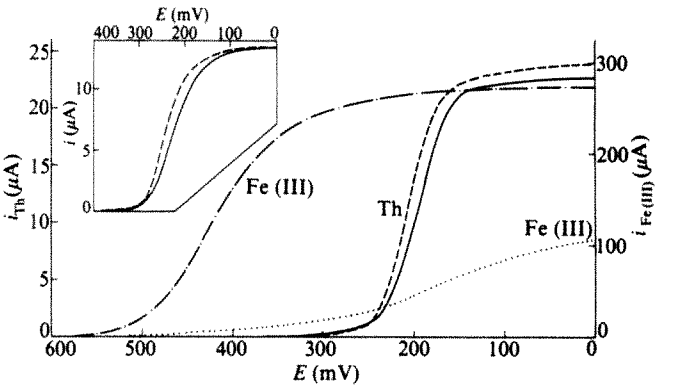


Fig. 4 Typical current–voltage curves for thionine (Th) and Fe(III) on an uncoated (– – – – – and – · – · – and a thionine coated Pt electrode (——— and .....). The inset shows similar current–voltage curves for disulphonated thionine.

in electrodes with lower electrochemical rate constants. For instance after coating for 1 h at 1.4 V the rate constant is about 1 cm ks^{-1}. Thus by choosing the coating voltage and time, the performance of the electrode can be controlled. We conclude first that on the coated electrode there is little difference between Pt and SnO_2 as the substrate surface. Second that the electrochemical rate constant is decreased for electrodes coated at higher voltages and for longer times. In particular there is a significant reduction in rate constant for electrodes coated at 1.4 V or above for periods longer than one hour.

Disulphonated thionine: photogalvanic cells have been extremely inefficient for the conversion of solar energy[11] because thionine is not sufficiently soluble to absorb the solar radiation close enough to the illuminated electrode[4]. We have recently synthesised 3,6-disulphonated thionine. The solubility is increased so that solutions ~10^{-1} M can be prepared and the visible spectrum shows no evidence for dimer formation. The disulphonated thionine cannot be coated onto the electrode in the same way as thionine. However, as shown in Fig. 4, the electrode kinetics of disulphonated thionine are very similar to those of thionine. Hence the thionine coated electrode can be used in a photogalvanic cell that operates with the more soluble disulphonated thionine.

Quinone: we investigated the reduction of quinone on the thionine coated electrode and found that the coating had practically no effect. A clean electrode and an electrode coated for 15 min at 1.4 V gave the same rate constant of 0.2 cm ks^{-1}. Hence thionine coating makes little difference to this typical organic redox system.

Fe(III): turning to inorganic couples, we find that the thionine coating makes a very significant difference to the electrode kinetics. We start with the reduction of Fe(III), which is the reaction that we wish to block in the photogalvanic cell.

Typical current–voltage curves for the reduction of Fe(III) are shown in Fig. 4. The coating produces first a shift of some 200 mV in the half wave potential and second the limiting current of the shifted wave is very much less than that on the untreated electrode. The variation of the limiting currents, i, of the shifted waves with rotation speed, W, are plotted in Fig. 5 according to the Koutecky Levich[12] equation:

$$\frac{nAF[\text{Fe(III)}]}{i} = \frac{0.65D^{-2/3}\nu^{1/6}}{W^{1/2}} + \frac{1}{k_7} \qquad (2)$$

where n is the number of electrons transferred; A, the electrode area; F, the Faraday constant; D, the diffusion coefficient of the electroactive species; ν, the kinematic viscosity; and k_7 the heterogeneous rate constant for the additional rate limiting step.

In equation (2) the term in the rotation speed, $W^{1/2}$, dominates the right hand side when the limiting current is controlled by mass transport. On the other hand the intercept of Fig. 5 gives the term in k_7 which corresponds to the control of the

limiting current by a kinetic process at the electrode which is independent of potential. Analysis of the current–voltage curves shows: first, that the coating has reduced the electrochemical rate constant by 10^{-2}; second that the transfer coefficient α is still $\frac{1}{2}$; and third that the limiting current is controlled by a second kinetic process described by k_7.

A reaction scheme of the following type would explain the data,

$$\text{Fe(III)} + L_{ads} \xrightarrow{k_7} \text{Fe(III)}L \xrightarrow[e]{k'} \text{Fe(II)}$$

where k_7 describes the formation of a complex between Fe(III) and coated leucothionine while k' describes an electron transfer with $\alpha \simeq \frac{1}{2}$. The fact that $\alpha \simeq \frac{1}{2}$ suggests that that the potential dependent rate constant is describing an electron transfer, even though the rate is much slower on a coated electrode compared to a clean electrode. The conductivity of the thionine film means that there cannot be large potential differences within the film and hence the main potential drop occurs at the thionine/water interface. We find that although Fe(III) has a different electrode kinetics on clean Pt (fast) and clean SnO_2 (slow), there is not much difference once the electrodes are coated. These results will be discussed further below.

$Fe(CN)_6^{4-}$: results for the oxidation of $Fe(CN)_6^{4-}$ in 0.4 M K_2SO_4 at *p*H 7 are similar to those for Fe(III). On a clean Pt electrode as expected the couple is reversible but on a coated electrode the wave is irreversible and again there is a second step. For an electrode coated at 1.1 V for 15 min we find $k_0'/\text{cm ks}^{-1} \simeq 0.6$ and $k_7/\text{cm ks}^{-1} \simeq 2.2$. It is very striking how the thionine coat has blocked this normally rapid electron transfer.

$Ru(bpy)_3^{3+}$: even more dramatic are the results for the reduction of $Ru(bpy)_3^{3+}$ in 0.5 M H_2SO_4 (where bpy is 2,2′ bipyridine). Results on a coated electrode are shown in Fig. 6. On a clean electrode $Ru(bpy)_3^{3+}$ is reduced at potentials <1.0 V, in a reversible wave. The thermodynamics for the reduction are favourable and the kinetics are rapid. However, on the coated electrode the transport limited current is not reached until 0.30 V. The current–voltage curve shows that there are two parallel processes. For potentials >0.40 V we find a small current which increases gradually as the potential decreases. A reasonably straight Tafel plot is obtained with a low value of $\alpha = 0.05$. At 0.4 V a second process takes over and the current rises rapidly to its transport limited value. Analysis of this part of the current–voltage curve gives $\alpha \simeq 2$. This high value of α cannot be caused by driving the electron transfer for the reduction of Ru(III). For that reaction we would expect (as was found for Fe(III)) that $\alpha = \frac{1}{2}$, and in any case α must be <1. We propose that the second process is the reaction of Ru(III) with L formed on the outside of the layer:

$$3H^+ + \underset{f}{\text{Th}} + 2e \rightleftarrows \underset{1-f}{\text{L}}$$

$$\underset{1-f}{\text{L}} + Ru(bpy)_3^{3+} \xrightarrow{k'} S^{\cdot} + Ru(bpy)_3^{2+} + 2H^+$$

The very small fraction of L ($\sim 2 \times 10^{-3}$) present when the second process takes over can be estimated from the Nernst equation (1) and the value of E_c' for 0.5 M H_2SO_4 where $E_c'/V = 0.270$. Figure 3 shows that the oxidation state of the thionine layer obeys the Nernst equation, and so we can calculate the current–voltage curve in Fig. 6 using the parameters derived from the analysis. Although our model for the second process fits the data very well, we do not yet have a detailed model for the slow process occurring at potentials >0.4 V. The very low value of α means that the process is not a straightforward electron transfer. This process may be similar to the k_7 step in the reduction of Fe(III). The potential range over which we can observe the k_7 process for Fe(III) is too restricted to be certain if k_7 depends slightly on potential or not.

Ce(IV): the reduction of Ce(IV) resembles that of $Ru(bpy)_3^{3+}$. Again two processes are evident. Following the same analysis as for $Ru(bpy)_3^{3+}$ we find that the steep part gives a curved Tafel plot with α varying between 1.4 and 2.0. The fact that these values are >1 suggests that we must again be seeing a reaction between L in the coat and Ce(IV).

Effect of coating voltages and coating times: Our interest in photogalvanic cells has led us to investigate the thionine and Fe(III) systems in more detail. We find that there is some reduction in the kinetic parameters the higher the coating voltage and the longer the coating time. We have found that there is a good correlation between the electrochemical rate constants for thionine or for Fe(III) and k_7. As these rate constants describe different processes we assume that the correlations arise through a general effect of the higher voltage or coating time on the coat itself. This affects all three processes, the reduction of thionine, the electrochemical step and the k_7 step in the reduction of Fe(III), to much the same extent. The higher coating voltages and coating times may cause this, for instance, by blocking off active sites on the surface.

Inorganic electron transfers: While the coat seems to have no drastic effect on the reduction of thionines or of quinone, single electron transfers to inorganic ions which normally proceed by outer sphere mechanisms are in all cases blocked by the coat. This is probably because the thickness of the coat prevents the direct tunnelling of electrons from the metal or SnO_2 to the solvated inorganic ion. For strong oxidising agents like Ru(III)

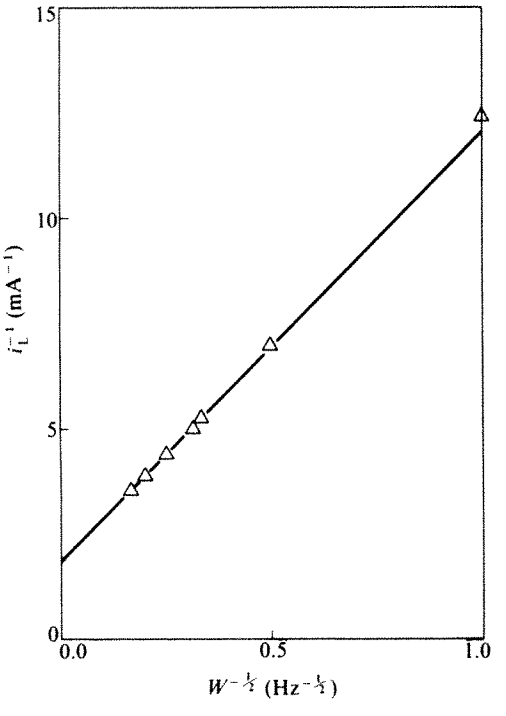


Fig. 5 Koutecky Levich plot, equation (2), for reduction of Fe(III) at thionine coated Pt electrode.

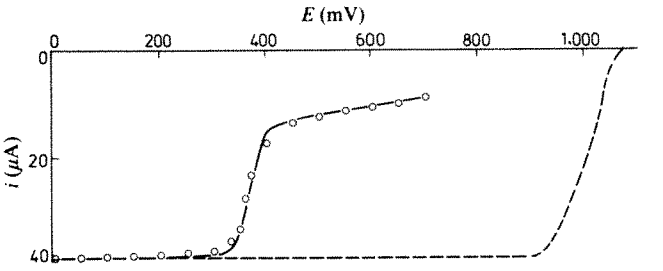


Fig. 6 Current–voltage curves for reduction of $Ru(bipy)_3^{3+}$ on a clean (– – – –) and a coated (○) Pt electrode. The solid line is the calculated curve for the two parallel processes discussed in the text.

and Ce(IV) the main path for reaction is apparently the oxidation of leucothionine on the outside of the coat. For Fe(III), because there is not such a strong thermodynamic driving force, the reaction between Fe(III) and L is 7 orders of magnitude slower than diffusion control. Hence we apparently have a two step mechanism of which one step is an electron transfer and we suggest that the other is the formation of a 'complex' between Fe(III) and leucothionine at the surface. Such complexes have been found in homogeneous reactions between Fe(III) and L (ref. 13). Note that the half wave potential for the reduction of Fe(III) is close to that of E' for thionine suggesting that the conversion of thionine to leucothionine in the coat may well be necessary for the reaction.

Implications for photogalvanic cells: Finally we return to the question of the selectivity of these electrodes for use in photogalvanic cells. Figure 4 shows that the thionine coated electrode does have the required selectivity. The coating leaves the electrode kinetics of the thionine couple sufficiently rapid to handle the photogenerated leucothionine[4] but it blocks the reduction of Fe(III). Thus an illuminated electrode working at a potential where it is oxidising leucothionine will only have a small current caused by the simultaneous reduction of Fe(III). Hence we conclude that the thionine coated electrode is an ideal illuminated electrode for the iron–thionine photogalvanic cell. The fact that it can be made so easily and that it is relatively stable are added advantages.

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Identification of the protein encoded by the transposable element Tn*3* which is required for its transposition

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*Protein products have now been identified which account for the entire coding capacity of the transposable element Tn*3*. Mutations in Tn*3* have allowed us to map the genes encoding each of these peptides and to identify their role in transposition. We have found that only a single Tn*3*-encoded peptide is required for transposition. Expression of this peptide is repressed by the product of a second gene, which is itself autogenously regulated.*

PLASMID-MEDIATED antibiotic resistance in bacteria has often been shown to reside on discrete DNA segments capable of transposition to other replicons in the cell (for review see refs 1–5). This event occurs in the absence of *rec*A-mediated recombination and does not require extensive regions of homology. One such transposable element, Tn*3* (refs 6–8) confers resistance to ampicillin by encoding for the production of the penicillin hydrolysing enzyme β-lactamase. This transposon is a particularly useful focus of study for the examination of the transposition process as a molecular event.

Analysis of mutants of the Tn*3* element[9,10] has resulted in a relatively simple map of the genetic organisation of the Tn*3* functions and structures necessary for its transposition and those related to the regulation of transposition frequency (Fig. 1). The element itself is about 5,000 base pairs in length. The 38 base pairs at each end are inverted and repeated sequences (IR)[11]. Mutations affecting either of the IR result in a transposition-deficient phenotype, and at least the left IR is required in *cis* (ref. 10). (The element will always be discussed in the orientation shown in Fig. 1.) The structural gene for the β-lactamase enzyme (*bla*) maps to the far right end of the transposon[9,10] (Fig. 1). Adjacent to the *bla* gene and bisected by the *Bam*HI restriction site, is the part of the transposon which we refer to as the 'regulatory region'. This region is known to consist of at least one gene, which we designate *tnpR*, which encodes a *trans*-acting 19,000-molecular weight (MW) peptide that represses the level of Tn*3* transposition[9,10,12,13]. Adjacent to the regulatory region and extending to the left IR is a 3000-base pair stretch of DNA which encodes one or more *trans*-acting functions necessary for Tn*3* transposition[9,10,12]. For convenience, we shall refer to this segment of the element as the 'transposase' region. This terminology, however, is only descriptive of the phenotype of mutations which saturate this region of the Tn*3* map; it is not meant to imply any particular enzymatic activity for the functions encoded by this region.

The genetic map of Tn*3* had been formulated from the analysis of two sets of Tn*3* mutants[9,10]. Both were the result of *in vitro* manipulation of the plasmid RSF1050, a small derivative of ColE1 into which Tn*3* had been transposed. The first set of RSF1050 mutants contained various-sized deletions having one or both endpoints within the transposon[9]. The newer collection was constructed by random *in vitro* insertion of an oligonucleotide containing an *Eco*RI restriction site into the Tn*3* element[10]. We will refer to this collection of mutants as '*Eco*RI-insertion mutants'. Many of the *Eco*RI-insertion mutants have now been sequenced (F. Heffron *et al.*, in preparation). It was found that, in addition to the insertion of the *Eco*RI site oligonucleotide, a small deletion or duplication of Tn*3* sequences occurred at the site of the insertion. However, none of these was greater than 50

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base pairs. The result is that many of the mutations shift the translational reading frame distal to the mutation. These would, therefore, be expected to have the same utility as frameshift mutations for mapping location and direction of gene transcription. Other *Eco*RI-insertion mutations have been found to retain the original reading frame and so will be expected simply to result in the addition of a few amino acids to the translation product at the point of the mutation.

These mutants were used to map the *tnpR* gene and study its regulation of transposition frequency. They have also allowed us to identify the peptide encoded by the 'transposase' region of the element.

Genetic analysis identifies only a single Tn*3* gene necessary for its transposition

Previous analyses of Tn*3* mutants have shown that all of the recessive transposition-deficient (Tnp$^-$) mutants are clustered in the transposase region of the element[9,10]. To establish a genetic basis with which to approach the biochemistry of transposition, we wished to determine the number of functions encoded by this region which are required for transposition to occur. Therefore, we initially attempted a complementation analysis of these Tnp$^-$ mutants.

To use the existing Tn*3* mutants, which were all isolated on the plasmid RSF1050, it was necessary to circumvent the problem of plasmid incompatibility when testing pairs of these mutants for complementation. Therefore, a number of Tnp$^-$ deletion or insertion mutants of Tn*3* were transposed by complementation by wild-type Tn*3* on to a plasmid compatible with RSF1050; either JCFL4 (F' *lac* $tra_{am}K4$)[14], or a non-colicinogenic derivative of the plasmid ColK (provided by D. Sherratt). These plasmids were then introduced into *rec*A$^-$ cells containing a conjugative plasmid and representative RSF1050Tnp$^-$ mutants. Strains containing each of the combination of Tn*3* mutants were tested for complementary transposition using the mating assay described previously[9,12]. In this manner, 5 deletion and 16 *Eco*RI-insertion mutants spanning the transposase region were tested in pairwise combinations for complementation. Data for representative Tnp$^-$ mutants are shown in Table 1. Although the assay was sensitive enough to detect transposition at a frequency of at least 1% of the wild type, these experiments failed to detect complementation between any of the pairs of mutants tested. These results indicate that Tn*3* encodes only a single *trans*-acting function in the transposase region, although other explanations could not be eliminated by these experiments.

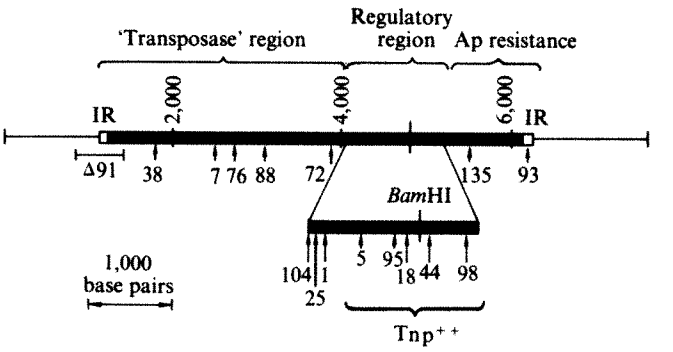


Fig. 1 Map of the Tn*3*-containing plasmid RSF1050[9,10]. The plasmid is shown as a linear molecule after cleavage at the *Eco*RI site in the ColEl portion of the molecule. The Tn*3* element is indicated by the bold line; ColEl-derived sequences are denoted by the thin line. The inverted and repeated termini of the element, labelled IR, are denoted by an open box. The Tn*3* element is divided into three sections based on the phenotype of mutations which map there, as explained in the text. Arrows (↑) denote location of *Eco*RT-insertion mutations[10]; each is identified by its pFH number. pFH7, pFH76 and pFH88 are Tnp$^-$; those mapping to the regulatory region are Tnp^{++}; pFH135 has lost the ability to confer resistance to ampicillin; pFH93 is a *cis*-dominant Tnp$^-$ mutation affecting the right IR, which is able to complement mutations in the 'transposase' region; mutation Δ91 is a deletion which removes about 300 base pairs of Tn*3*, including the left IR (F. Heffron *et al.*, in preparation).

Identification of Tn*3*-encoded peptides in minicells

We next attempted to confirm our genetic evidence for a single function encoded by the Tn*3* transposase region by visualising its protein products directly. Dougan *et al.*[13] have reported the

Table 1 Complementation of Tn*3* mutants

		RSF1050 derivative					
	JCFL4 derivative*	A RSF1050$^+$	B pFH72	C pFH88	D pFH7	E pFH38	Comments
1	JCFL4	(—)	0	0	0	0	Negative control. The level of transportation of Tn*3* from the Tnp$^-$ mutants of RSF1050 was below the limit of detection (0.5×10^{-6} or a normalised value of 0.004)
2	JCFL4::Tn*3*-135$^+$	1.0	0.3	0.1	0.4	0.1	Positive control. Tnp$^-$ mutants were complemented in *trans* by a transposition proficient Tn*3* element on JCFL4†
3	JCFL4::Tn*3*-72	1.0	0.0009	0.006	0.001	0.003	The Ap^r transconjugants reported in columns B–E had received the JCFL4 derivative and represent reversion or supression of the *traK4* mutation on the F'*lac*. No transposition on to R388 was detected
4	JCFL::Tn*3*-38	1.0	0.002	0.001	0.007	0.003	

Complementation experiments were attempted using strain M1653 (*rec*A$^-$, Su^0) containing the conjugative plasmid R388 (trimethoprim and sulphonamide resistant) plus representative Tnp$^-$ mutants of RSF1050. The plasmid JCFL4 (F'*lac traK4*) into which representative Tnp$^-$ mutants of Tn*3* had been transposed was then introduced into these strains. JCFL4 was not transmissible from the Su^0 strain, M1653. Therefore, transposition (by complementation *in trans*) of either of the mutant Tn*3* elements into the co-resident conjugative R388 plasmid could be measured by selecting for the transfer of Ap^r into a suitable recipient in a 4 h mating. The frequency of transposition was: no. of Ap^r transconjugants/total no. of transconjugants. Representative data are presented for four Tnp$^-$ mutants of RSF1050 (columns A–E) and two Tnp$^-$ mutants of Tn*3* on JCFL4 (lines 3 and 4). The values entered into the table were normalised to the frequency of transposition of wild-type Tn*3* from RSF1050 (1.3×10^{-3}) which was given a value of 1.0.

* Locations of the respective mutations in Tn*3* are shown in Fig. 1.

† Tn*3*-135 (ref. 9) is a Tn*3* derivative which has a determinant encoding tetracycline resistance cloned into and inactivating the ampicillin resistance gene. Transposition functions have not been detectably altered. Use of this element made it possible to directly select for the complemented transposition of the co-resident Tnp$^-$ (Ap^r) element on RSF1050.

use of *Escherichia coli* minicells[15] containing RSF1050 or representative RSF1050 deletion mutants to identify polypeptides encoded by defined regions of the Tn*3* element. These investigators reported, however, that no protein encoded by the transposase region of the element was synthesised in detectable amounts by minicells. The functions encoded by this region are presumably repressed by the wild-type element[10,12].

E. coli minicells were also used here, but advantage was made of several recently isolated transposition-proficient mutants of Tn*3* which had lesions confined to the regulatory region of the element[10]. These mutations resulted in a 10–50-fold increased frequency of transposition of the mutant element, and were recessive to wild-type Tn*3* for this behaviour (ref. 10, R. E. G., unpublished observation). The phenotype of these mutants, designated Tnp^{++}, suggests that they possess mutations which allow increased expression of the function(s) encoded by the transposase region.

Five of the Tnp^{++} mutants of RSF1050 used in this study were from the collection of *Eco*RI-insertion mutants isolated by Heffron *et al.*[10] (see Fig. 1). The remaining Tnp^{++} mutant was prepared by enzymatic manipulation of the *Bam*HI restriction site of Tn*3*. *Bam*HI cleaved RSF1050 only once, at a site in the Tn*3* regulatory region, generating a linear molecule with single-stranded 5′ protruding ends. These ends were made double stranded by polymerising the complementary strand with T4 DNA polymerase. This manipulation introduced a 4-base pair duplication at the *Bam*HI site: G$^{\downarrow}$GATCC to GGATC*GATC*C. Consequently, a frameshift mutation was introduced into the gene encoded at that point. As the mutant sequence was no longer cleaved by *Bam*HI, the mutant element will be referred to as a *Bam*HI^{0} mutant.

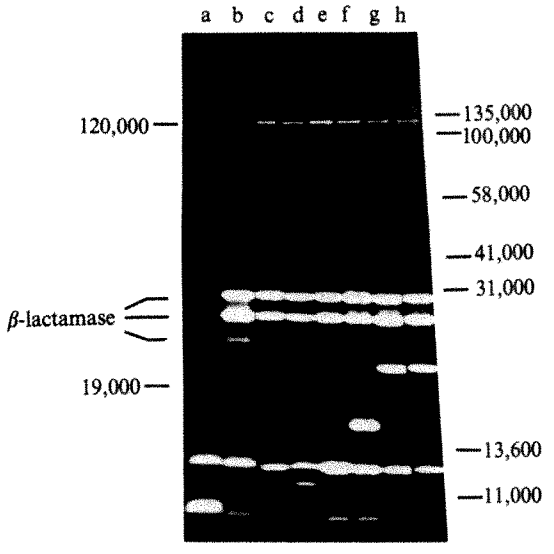


Fig. 2 Identification of Tn*3* encoded peptides. Minicells were purified[22] from cultures of *E. coli* DS410[23] containing the plasmid RSF1050 or Tnp^{++} mutants of RSF1050. Proteins were labelled in M9 minimal media with 30 μCim^{-1} of ^{35}S-methionine for 45 min. Minicells were collected and lysed by boiling in 80 μl of cracking buffer (50 mM Tris-HCl, *p*H 6.8, 1% SDS,, 1% β-mercaptoethanol, 5% glycerol). The proteins were precipitated by addition of 1.2 ml of acetone for 20 min at room temperature. The precipitate was pelleted and resuspended in final sample buffer (0.0626M Tris, *p*H 6.8, 3% SDS, 5% β-mercaptoethanol, 10% glycerol), and boiled 3 min. Approximately, 60,000 c.p.m. were applied to each well of a 10–18% gradient SDS polyacrylamide gel[19], and electrophoresed at 130 V until the dye reached the bottom of the gel. Gels were prepared for fluorography as described[24] and exposed for 18 h at −70 °C. Samples were prepared from minicells carrying the following plasmids: Lane a, pMB8, which is the ColEl derivative into which Tn*3* was transposed to make the plasmid RSF1050; lane b, RSF1050, (pMB8 :: Tn*3*, wild type); lanes c–h Tnp^{++} mutants pFH5, pFH95, a *Bam*H1^{0} mutant of RSF1050, pFH44, pFH18 and PFH98, respectively. Location of each mutation is shown in Fig. 1. Migration of molecular weight standards is shown on the right margin. Standards were: *E. coli* β-galactosidase, MW 135,000; phosphorylase *b* (rabbit muscle), MW 100,000; catalase (beef liver), MW 58,500; alcohol dehydrogenase (horse liver), MW 41,000; DNase 1 (bovine pancrease), MW 31,000; RNase A (bovine pancrease), MW 13,600; cholera toxin B subunit, MW 11,000. The 120,000 and 19,000-MW peptides from Tn*3* and the three forms of the β-lactamase gene product are identified in the left margin.

We have examined the Tn*3* specific polypeptides synthesised in *E. coli* minicells carrying each of the RSF1050 Tnp^{++} mutants (Fig. 2). Peptides encoded by wild-type Tn*3* can be identified by comparing lane a, pMB8, with lane b, RSF1050 (pMB :: Tn*3*). The β-lactamase gene product, which is known to undergo post-translational processing[16,17] resolves into three discrete bands with our electrophoresis conditions (also see ref. 13). Minicells carrying wild-type Tn*3* (lane b) also synthesise a 19,000-MW peptide. This peptide was shown by Dougan *et al.*[13] to be encoded by the Tn*3* regulatory region by the gene we designate here as *tnpR*. They also observed the correlation that Tn*3* mutants having the phenotype of increased transposition frequency failed to produce this 19,000-MW peptide.

In contrast to the wild-type element, the 19,000-MW peptide is not synthesised in minicells carrying any of the Tnp^{++} mutants (Fig. 2, lanes c–h). Minicells containing pFH5, PFH95, a *Bam*HI^{0} mutant of Tn*3*, or pFH44 (Fig. 2, lanes c–f) each produced an additional prominent peptide which we interpret to be truncated forms of the 19,000-MW *tnpR* gene product. The truncated peptide encoded by pFH5 has eluted off the gel, but those encoded by pFH95, the *Bam*HI^{0} mutant, and pFH44 migrated with apparent molecular weights of 11,500, 12,500 and 15,500, respectively. The peptide from the *Bam*HI^{0} mutant co-migrated with a peptide encoded by the pMB8 portion of RSF1050 in these electrophoretic conditions. The size of the truncated peptide encoded by each of the mutants is related to the map position of the mutation in such a way as to suggest that transcription of *tnpR* proceeds rightward and that the initiation of translation corresponds to the 4,200 base pair coordinate of RSF1050 (Fig. 1). This is between the mutation in pFH1 and pFH5, supporting the observation that mutation 1 does not lie in the *tnpR* gene itself (see below).

Minicells containing the Tnp^{++} mutants pFH18 and pFH98 also fail to synthesise a 19,000-MW peptide (Fig. 2, lanes g, h). However, each encodes a new peptide slightly larger than this. These peptides have been shown to be highly related to the wild-type protein by comparison of the fragments generated by partial chymotrypsin or *Staphylococcus aureus* V8 protease cleavage[18] of each of the respective peptides (data not shown). The nucleotide sequence surrounding mutations 18 and 98 have been determined (F. Heffron *et al.*, in preparation). Mutation 18 was found to preserve the transcriptional reading frame of the distal portion of the *tnpR* gene, but had an insertion at the site of the mutation sufficiently large to account for the synthesis of the observed peptide. A somewhat similar explanation accounts for the observed peptide encoded by pFH98.

Dougan *et al.*[13] have previously observed that truncated forms of the 19,000-MW peptide from *tnpR* mutants of the Tn*3* were overproduced in minicells. Results shown in Fig. 2 tend to corroborate that observation, at least for pFH44, pFH18, pFH98 and the *Bam*HI^{0} mutant. As measured by the density of the band image on fluorograph negatives, considerably more radioactivity was incorporated into the mutant *tnpR* products than into the wild-type 19,000-MW peptide. It seems, therefore, that the *tnpR* gene product represses the level of its own expression, as well as repressing the level of transposition.

Interestingly, the fluorograph in Fig. 2 also clearly shows that minicells containing any of the Tn*3* mutants which affect the 19,000-MW peptide (lines c–h) synthesise a previously undetected high molecular weight peptide. This peptide migrates between β-galactosidase (MW 135,000) and phosphorylase *b* (MW 100,000) in 7.5% SDS polyacrylamide gels[19] and has an apparent molecular weight of 120,000. Disregarding the possibility of overlapping genes, the transposase region of the element is just large enough to encode a 120,000-MW protein, and is the only such stretch of DNA on the transposon for which no other protein product has been identified.

We have examined the effect of Tnp^{-} mutations on the synthesis of this peptide to determine if it was, in fact, the product of the transposase region. We have already shown that

this protein is only made in detectable quantities by transposons which have mutations in the *tnpR* gene. Therefore, representative Tnp⁻ deletion and *Eco*RI-insertion mutants of Tn*3* were also made *tnpR*⁻ by introducing a 4 base-pair insertion at the *Bam*HI site, as described above. The proteins encoded by these mutant elements were labelled in minicells and separated on SDS polyacrylamide gels (Fig. 3).

As expected of *Bam*HI⁰ mutants, each of the minicell strains produced the characteristic 12,500-MW truncated *tnpR* product. None of these doubly mutant elements directed the synthesis of the 120,000-MW peptide, however (Fig. 3, lanes c–f). Furthermore, only mutations in the transposase region abolished synthesis of this peptide; *Bam*HI⁰ mutants of elements having *Eco*RI-insertion mutations elsewhere in the element, either in the β-lactamase gene (mutant 135) or in the right IR (mutant 93) continued to direct the synthesis of the 120,000-MW peptide (data not shown). These results indicate that the transposase region itself encodes the 120,000-MW peptide expressed in the *tnpR*⁻ mutants of Tn*3*. We have designated the gene encoding this peptide *tnpA*.

The *Eco*RI-insertion mutants used in Fig. 3 were selected because DNA sequencing had shown that they led to a shift in the transcriptional reading frame of the *tnpA* gene (F. Heffron *et al.*, in preparation). Thus, they could be used to determine the location of the ends of the *tnpA* gene and the direction of its transcription. Minicells containing *Bam*HI⁰ mutants of pFH7, pFH76[10], and pFH88 (lanes d–e) synthesised unique 60,000, 52,000 and 37,000-MW peptides, respectively. The molecular weight of these unique peptides correlated with the map position of the mutations in such a way as to suggest that the *tnpA* gene is transcribed leftward on the Tn*3* map in Fig. 1. Assuming that an amino acid has an average MW of 110, it was deduced that the beginning of translation of the *tnpA* gene product corresponds approximately to the 4,100 base pair coordinate of RSF1050, which in turn places the distal end of the gene very close to the left IR. The orientation of the *tnpA* gene was also deduced from the effect of the deletion Δ91 on the synthesis of the 120,000-MW peptide. Δ91 deletes ~300 base pairs of the left end of Tn*3* including the left IR (Fig. 1). This deletion reduced the molecular weight of the peptide from 120,000 to 105,000, presumably by removing the distal portion of the gene. It is interesting to contrast the phenotypes of Tn*3* elements having the Δ91 deletion with that of the *Eco*RI-insertion mutant pFH93. Mutant Δ91 removes the left IR; mutation 93 interrupts the right IR (Fig. 1). Both are *cis*-dominant Tnp⁻ mutations, presumably because both the left and right IR are required for transposition. Mutation 93 did not affect the *tnpA* gene product, and was found to complement *tnpA* mutants of Tn*3* (data not shown). Deletion mutant Δ91, however, will not complement over Tnp⁻ mutants, presumably because of the removal of the distal portion of the *tnpA* gene. We have also examined three additional transposition-proficient *Eco*RI-insertion mutants, pFH104, pFH25 and pFH1, which map just to the left of the Tnp⁺⁺ mutations (Fig. 1). The peptides synthesised from minicells containing these mutant plasmids appear identical to those encoded by the wild-type element (data not shown). In addition to the β-lactamase peptides, each of the mutant plasmids directs the synthesis the 19,000-MW product of the *tnpR* gene, but not detectable amounts of the *tnpA* gene product. Expression of the 19,000-MW peptide is consistent with the observation that these three mutants retained the ability to repress the frequency of transposition of Tnp⁺⁺ (that is, *tnpR*⁻) mutants. It also supports our earlier placement of the proximal end of the *tnpR* gene between the mutations in pFH1 and pFH5. However, whereas pFH1 transposed at near wild-type levels, mutants pFH104 and pFH25 each transposed at an elevated frequency, intermediate between wild-type and the Tnp⁺⁺ mutants, and were apparently repressed by a wild-type element (R.E.G., unpublished observation). The observation that pFH25 and pFH105 transposed at an elevated frequency, even though functional *tnpR* gene product was being produced, was unexpected. We do not believe, however, that these mutants contradict our scheme for the regulation of Tn*3* transposition frequency. Indeed, DNA sequence analysis of these mutations (F. Heffron, *et al.*, in preparation) indicated that these mutations actually affect the proximal end of the *tnpA* gene. If this is the case, then the increased transposition frequency may reflect an alteration in the activity of the *tnpA* gene product, rather than in its regulation.

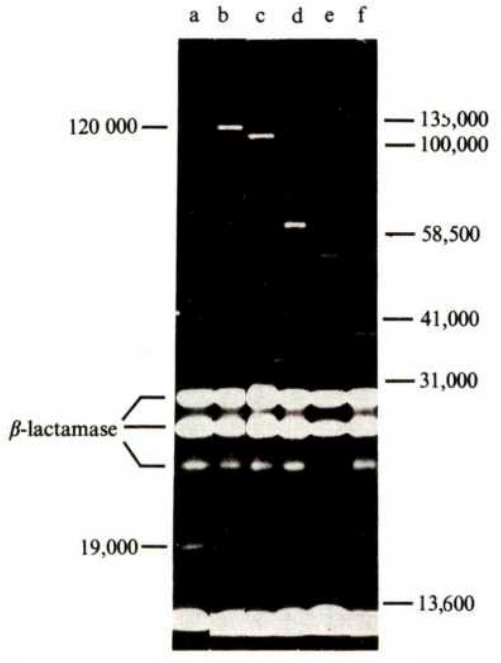


Fig. 3 Peptides synthesised in minicells containing transposition-deficient mutants of Tn*3*. Minicells were isolated, labelled and prepared for electrophoresis as in Fig. 2. Extracts were electrophoresed through an 11% SDS polyacrylamide gel at 100 V. Lane a, wild-type RSF1050; lane b, *Bam*HI⁰ mutant of RSF1050. Lanes c–f, RSF1050 derivatives which have a *Bam*HI⁰ mutation, as well as a mutation in the 'transposase' region of Tn*3*. The 'transposase' region mutations are: lane c, deletion 91; lane d, *Eco*RI-insertion mutation 7; lane e, *Eco*RI-insertion mutation 76, lane f, *Eco*RI-insertion mutation 88. Location of these mutations in Tn*3* is shown in Fig. 1. Molecular weight standards are those listed in Fig. 2 legend.



Fig. 4 Map Tn*3* showing location of its three genes. Arrows indicate direction of transcription. The size of the protein product of each gene is also indicated. Location of the *bla* gene and direction of its transcription are based on Heffron[9] and Sutcliffe[16]. Three peptides are associated with the β-lactamase gene in minicell extracts[14]. The largest of these, with MW of 29,000 when compared to our standards, is presumably the unprocessed *bla* gene product[16,17].

Conclusion

Tn*3*-specified proteins have now been identified which account for the entire coding capacity of the transposon. Our data indicate the presence of three genes encoded by the element as outlined in Fig. 4. The *bla* gene, encoding the β-lactamase enzyme, is situated at the right end of the element and is transcribed rightwards[9,16]. The remaining two genes, *tnpA* and *tnpR*, begin near the centre of the transposon and are transcribed in opposite directions. The position of these genes is consistent with the genetic organisation of the element as deduced from the DNA sequence of the element (F. Heffron *et al.*, in preparation).

The *tnpA* gene extends from its initiation near the centre of the element all the way to the left IR. All of the known recessive Tnp⁻ mutations of Tn*3* map in this gene. The existence of only a

single gene encoded by the transposase region is also supported by the observation reported here that recessive Tnp⁻ mutants appear to fall into a single complementation group. The product of the *tnpA* gene has been identified as a 120,000-MW peptide, which is expressed in detectable quantities by minicells containing only *tnpR*⁻ mutants of Tn*3*. As this peptide is required for transposition, its overproduction by *tnpR*⁻ mutants may account for the Tnp⁺⁺ phenotype of such mutants. Although necessary for transposition, no enzymatic function has yet been attributed to this peptide, and its possible interaction with host proteins has not been established.

The product of the *tnpR* gene is a 19,000-MW peptide. Our data are consistent with a dual regulatory role for this gene product. First, it appears to repress the level of *tnpA* expression. Overproduction of the 120,000-MW *tnpA* gene product by *tnpR*⁻ mutants presumably accounts for the elevated transposition frequency observed for these mutants. Second, the *tnpR* gene seems to regulate the level of its own expression. The mutant *tnpR* gene products are typically overproduced relative to the wild-type product. Regulatory proteins having such dual roles have also been observed for the *araC* gene[20] and *cI* gene of bacteriophage[21].

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Transposition protein of Tn*3*: identification and characterisation of an essential repressor-controlled gene product

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Fusions that bring lac *gene expression under the control of transcriptional and translational signals within the Tn*3 *element have been used to identify and characterise a Tn*3*-encoded 'transposase'* (Tnp) *peptide of MW 100,000 essential for transposition. The gene specifying this product is regulated by the Tn*3 *repressor protein and is part of a bidirectional genetic unit that includes the repressor gene.*

Tn*3* is one of a class of genetic elements capable of transposition between DNA segments having no sequence homology with each other or with the transposable element itself[1]. This element encodes functions that affect its own transposition[2,3] as well as a β-lactamase gene that renders host bacterial cells resistant to the antibiotics penicillin and ampicillin[4].

Recently, we have used Tn*3*–*lac* gene fusions to identify and analyse regulatory mechanisms that control the transposition of Tn*3* (refs 5–7). Plasmids containing both Tn*3* and *lac* were constructed and deletions that fuse the *lac* genes to Tn*3* sequences were introduced, bringing expression of *lac* under control of transcriptional and translational signals within the transposable element. Such experiments have shown that a DNA region within Tn*3* that is known to influence its transposition frequency[2,3,8] encodes a peptide of molecular weight 21,000 (21K) that represses transposition of Tn*3* and acts at the level of transcription to regulate its own synthesis[7].

During our studies of the Tn*3*-encoded repressor gene, we observed a prominent increase in the synthesis of a 100K peptide in Tn*3* mutants that fail to synthesise a functional repressor, suggesting that the gene specifying this peptide might also be regulated by the repressor. Here we report the use of *lac* gene fusions to identify and characterise the gene encoding the 100K peptide. Our findings indicate that this peptide is a repressor-controlled product required for transposition of Tn*3* and that its gene is transcribed in a divergent direction from the adjacent repressor gene.

Identification of a Tn*3*-encoded 100K peptide

Gel electrophoresis (Fig. 1*a*) of extracts from ^{35}S-methionine-labelled minicells[9] containing a nonsense (R49) or missense (R102) mutation in the repressor peptide showed a large band having a calculated molecular weight of 100K for both types of repressor-minus mutants; little or no such peptide was seen in extracts of minicells containing the wild-type (repressed) Tn*3*.

The location and extent of the gene encoding the 100K peptide were identified using the pMC1102 and pMC1104 plasmids (Fig. 2), which carry Tn*3* elements that have deleted internal sequences, and the pMC823 plasmid, which has deleted all of Tn*3* to the right of the sixth codon of the repressor gene (ref. 7; see also Fig. 4 below). The pMC1102 and 1104 plasmids include the R49 amber mutation in the Tn*3* repressor gene. As seen in Fig. 1, pMC1102 (Δ*Bcl*I) and pMC1104 (Δ*Pst*I) (Fig. 1*b*) do not encode the 100K peptide whereas pMC823 does (Fig. 1*c*). This locates the 100K peptide gene between the left end of Tn*3* (IRa) and the beginning of the repressor gene, which is the only region of Tn*3* retained on pMC823. From the size of the 100K peptide, it can be calculated that almost this entire segment must be used to encode the peptide (Fig. 2). The orientation of the gene for the 100K peptide can be inferred from the size of the 66K truncated peptide (Fig. 1*b*) formed by the pMC1104 (Δ*Pst*I) deletion plasmid. The 66K peptide is too large to be encoded entirely to the left of Δ*Pst*I and too small to

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span it in frame. Thus, the NH_2-terminus of the 66K peptide can be assigned to the right of Δ*Pst*I, thus implying a right-to-left orientation for the gene. This assignment is consistent with the absence of a detectable truncated peptide for the pMC1102 (Δ*Bcl*I) deletion plasmid which retains only a very small part of the right end of the 100K gene.

Assignment of the gene for the 100K peptide gene to a location at the left end of Tn*3* (Fig. 2) exhausts the coding capacity of the transposon, unless overlapping or very small genes are present. Tn*3* is thus seen to encode three peptides—a β-lactamase[4,10], a self-regulated repressor protein[7], and the repressible 100K peptide.

The Δ*Pst*I and Δ*Bcl*I deletions, both of which fail to synthesise the 100K peptide (Fig. 1), do not undergo transposition at a detectable frequency even when coupled to a mutation (R49) in the Tn*3* repressor gene that otherwise leads to a 14-fold increase in the frequency of transposition (data not shown). This observation, together with earlier evidence[2,3] that deletions or insertions in the vicinity of these endonuclease cleavage sites prevent transposition, suggests that the repressor-controlled 100K peptide (hereafter termed 'transposase' or *Tnp* gene product) carries out functions essential to the transposition process.

The effects of repressor on synthesis of the Tnp protein are shown in Table 1. As seen in the table, missense and amber mutations in the Tn*3* repressor lead to a 10- and 60-fold increase in production of a missense repressor peptide or a nonsense truncated peptide respectively. The greater extent of derepression observed with the nonsense mutation (R49), which yields a truncated repressor peptide[7], compared with the missense mutation (R102), which results in the formation of a full-length peptide having a presumed amino acid substitution, could be explained if the peptide product of the missense mutant is partially functional. This interpretation is consistent with the observed fivefold increase of the Tnp peptide in the missense mutant versus the eightfold increase of the Tnp peptide in the two nonsense mutants examined.

Use of *lac* gene fusions for study of regulation of the Tnp protein

Introduction of a promoter-less *lac* gene segment within the *Tnp* gene provides a convenient assay for studying *Tnp* gene expression. In these experiments the segment of the *Tnp* gene between the two *Bcl*I sites of Tn*3* carrying the R49 mutation in the repressor gene was replaced (in the orientation shown for the pMC1103 plasmid, Fig. 2) by a *lac* gene-derived sequence (endonuclease-generated fragment 903 *lac*, ref. 11) that lacks a promoter but contains a translational start signal for the *lacZ* structural gene, which encodes the enzyme β-galactosidase. Expression of *lac* by pMC1103 (Table 3, line 1) verifies the proposed direction of transcription of the *Tnp* gene and demonstrates that the transcriptional start for this gene is located to the right of the $Bcl\text{I}_b$ site. When a plasmid carrying a wild-type Tn*3* (that is, the pACYC999 plasmid) was placed *in trans*, a decrease in β-galactosidase to 15% the level specified by the pMC1103 plasmid was observed; there was no change in the level of *lac* gene expression when a Tn*3* element carrying the R49 amber mutation was introduced *in trans*, demonstrating directly that transcription of the segment of the *Tnp* gene linked to *lacZ* in pMC1103 is controlled by the repressor substance.

Suppression of the R49 amber mutation in the Tn*3* repressor gene by a bacteriophage carrying an amber suppressor (λsupF, Table 2, line 4) resulted in decreased synthesis of β-galactosidase, providing additional verification that the Tn*3* repressor substance regulates expression of the *Tnp* gene. The degree of suppression of the amber mutation observed in these

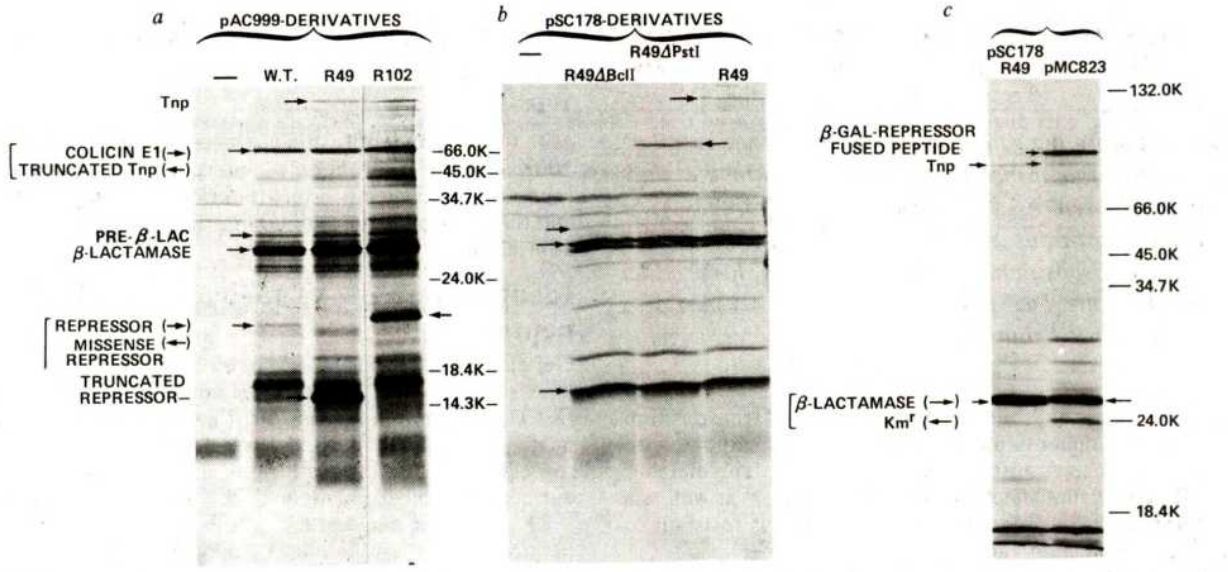


Fig. 1 Autoradiograms showing ^{35}S-labelled polypeptides synthesised by *E. coli* minicells containing plasmids carrying Tn*3* or its mutant derivatives. Media used[32], purification of minicells[23], and labelling[23] and electrophoresis[24] of peptides in polyacrylamide-SDS gels (15%, panels *a* and *b*; 10%, panel *c*) have been described. Molecular weight standards (between panels *a* and *b*; right-side, panel *c*) were lysozyme (14,300), β-lactoglobulin (18,400), trypsinogen (24,000), pepsin (34,700), ovalbumin (45,000), bovine plasma albumin (66,000), and cross-linked bovine plasma albumin (panel *c* only, 132,000). In panel *a* are shown the patterns of peptides from the minicell strain lacking a plasmid (−) and from minicells carrying the pACYC999 (ColE1::Tn*3*) plasmid or its derivatives, containing the R49 or R102 repressor mutations. Arrows in this panel (and in *b* where appropriate) indicate the positions of the 100K peptide encoded by Tn*3* (Tnp), colicin El (→), the 28K β-lactamase protein and its 30K precursor (pre-β-*lac*)[9,28] and the wild-type, nonsense truncated (R49), and missense (R102) Tn*3* repressor peptides. The Tn*3* deletion plasmids pMC1102 (Δ*Bcl*I) and pMC1104 (Δ*Pst*I) (panel *b*) are derivatives of the pMC1097 plasmid, which is a pSC178 (pSC101::Tn*3*) derivative that carries the R49 amber mutation in the Tn3 repressor gene. pMC1102 and pMC1104 were constructed from pMC1097 DNA by removing internal *Bcl*I and *Pst*I endonuclease-generated fragments of Tn*3* (Fig. 2) by digestion with the respective enzymes, ligation[29], transformation[30], and selection for Ap^RTc^R in the χ1488M minicell-producing strain (obtained from Roy Curtiss III; see also ref. 7). Selection for resistance to Ap ensures that the pMC1104 plasmid has lost only the left *Pst*I fragment of Tn*3*. An additional arrow (←) in panel *b* indicates the position of a 66K truncated Tnp peptide synthesised by the pMC1104 (Δ*Pst*I) deletion mutant. Panel *c* shows patterns for the pSC178-R49 (pMC1097) and pMC823 (ref. 7) plasmids. The latter is a derivative of the pSC105::Tn*3* plasmid in which the *lac* genes have been fused to the Tn*3* element such that the 6th amino acid of the Tn*3* repressor is joined to the 21st amino acid of the *lac*Z gene product, resulting in a fused peptide. The kanamycin-resistant pSC105::Tn*3* plasmid was used as a replicon to construct pMC823 (ref. 7). This plasmid was used to provide an additional selective marker, since the β-lactamase gene of Tn*3* and the Tc-resistant gene of pSC101 are both lost by deletion of the *Bam*HI-generated DNA fragment containing the IRb terminus of Tn*3* to yield pMC823. Arrows in panel *c* indicate for pMC823 the location of the β-galactosidase-repressor fused peptide, the kanamycin resistance gene product (←), and the Tnp peptide. The positions of the 100K Tnp peptide and the β-lactamase protein (→) are shown for the pSC178-R49 plasmid.

experiments (30%) is consistent with the previously reported efficiency of amber suppressors (25–65%)[12]. Analogous experiments carried out in minicells (data not shown) demonstrated a 70% reduction in the amount of the 100K Tnp peptide encoded by the pMC823 plasmid (which has deleted the entire repressor gene, see above) when the pACY999 plasmid, which carries a wild-type repressor gene, was placed *in trans*.

The level of expression of the Tnp–lac hybrid peptide encoded by the pMC1103 plasmid was found to be affected by temperature (5,217, 2,895 and 1,766 units of β-galactosidase at 30, 37 and 42 °C, respectively). These observations are consistent with the previously observed effects of temperature on translocation itself[13,14], and suggest that temperature may affect translocation frequency by a mechanism that controls the efficiency of transcription of the *Tnp* gene.

In the course of these experiments, we detected an additional transcriptional start signal on Tn*3* using the pMC945 plasmid (Fig. 2) which was constructed by insertion of the 903 *lac* sequence into the single *Bam*HI site of the transposon; the orientation of the inserted fragment (as determined by gel analysis, unpublished data) suggests that *lac* gene expression encoded by the pMC945 plasmid results from the presence of a transcriptional start signal located towards or past the carboxy-terminal end of the repressor gene and initiating leftward transcription through the repressor gene. We have not characterised this putative promoter further, and do not know whether it has a biologically significant role. No additional Tn*3* peptide assignable to this region has been identified in minicells. The promotor detected by insertion of the same 903 *lac* fusion fragment into the *Bam*HI site in the opposite orientation (that is, the pMC959 plasmid, Fig. 2) accomplishes expression of the Tn*3* repressor gene and is repressible by a wild-type Tn*3* *in trans* as expected (see ref. 7).

Localisation of the regulatory signals for the *Tnp* gene and determination of translational reading frame

The location and direction of reading of the repressor-controlled gene encoding the Tnp peptide suggested that its promoter is located near the promoter for the repressor gene, which is transcribed in the opposite direction. To investigate the genetic control of the *Tnp* gene and to identify more precisely where transcription and translation of this gene begin, we used

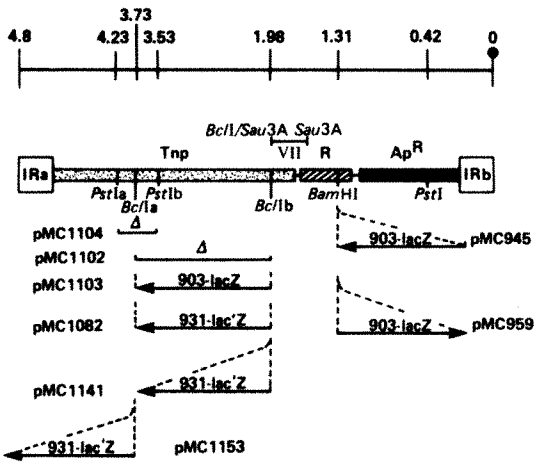


Fig. 2 Schematic map showing structure of *lac* insertions into Tn*3*. *Tnp* is the transposase gene, R the repressor gene, and Ap the β-lactamase gene. IRa and IRb are the inverted repeat termini of Tn*3*. The kilobase co-ordinates of restriction endonuclease cleavage sites discussed in the text are indicated; additional *Sau*3A sites within Tn*3* are not shown. Kilobase coordinates are given relative to the right termini of Tn*3* at IRa. The position of *Sau*3A fragment VII, which contains promoters and translational start signals for genes that are encoded in divergent directions is shown; the left terminus of this fragment is also the $BclI_b$ cleavage site. The segments deleted in the pMC1104 plasmid (Δ*Pst*I) and pMC1102 plasmid (Δ*Bcl*I) are indicated. The 38-base-pair-long inverted repeat termini of Tn*3* (IRa and IRb) are not drawn to scale. The underlying arrows indicate the direction of insertion of 7-kilobase *Bam*HI-generated fragments containing *lacZ* and *lacZ* genes that lack promotors[11]. 903-*lacZ* is missing only the promotor for *lacZ* transcription while *931-lac'Z* lacks both the promotor and a translational start site. The 931-*lac'Z* segment starts with the same *lac* sequence shown in Fig. 3. Insertions of *lac* gene fragments in the *Bam*HI site of Tn*3* were obtained by transformation of *E. coli* strain pMC1050 with ligated mixtures of one of the *lac* fragments and *Bam*HI-cleaved pACYC999 DNA, which contains only one *Bam*HI site in Tn*3* and none within the ColEl segment. *lac* insertions in the *Bcl*I sites were obtained by cloning the *lac* fragments into partially *Bcl*I-cleaved pSC101::Tn*3* R49 DNA (the pMC1097 plasmid) which has two *Bcl*I sites within Tn*3* and none in the pSC101 segment. The *Bam*HI and *Bcl*I enzymes yield identical complementary protruding ends. DNA to be cleaved by *Bcl*I was grown in a *dam*⁻ *dcm*⁻ methylase-deficient strain since methylation of DNA synthesised in *E. coli* K12 precludes *Bcl*I cleavage by the *Bcl*I enzyme.

Table 1 Effect of repressor on synthesis of the Tnp protein

Experiment 1

Plasmids	β-Lactamase (c.p.m.)	Normalisation ratio	Repressor (c.p.m.)	Transposase (c.p.m.)
(1) None	(102)*	—	(56)	(76)
(2) pACYC999 (ColEl::Tn*3* R⁺)	12,880	1.0	383†	105†
(3) pACYC999 R49 (ColE1::Tn*3* R49)	12,440	1.0	23,873†	804†
(4) pACYC999 R67 (ColE1::Tn*3* R67)	14,340	0.9	—	824†
(5) pACYC999 R102 (ColE1::Tn*3* R102)	21,892	0.6	3,760	507†

Experiment 2

Plasmids	Truncated repressor (c.p.m.)	Normalisation ratio	Transposase (c.p.m.)
(6) None	(381)	—	(92)
(7) pACYC999 R49	45,185	1.0	4,623†
(8) pMC1097 (pSC101::Tn*3* R49)	15,966	2.8	1,963†
(9) pMC1104 (pSC101::Tn*3* R49 ΔPst)	9,421	4.8	7,449(t)† (138)
(10) pMC1102 (pSC101::Tn*3* R49 ΔBc1)	9,445	4.8	(74)

The conditions for isolation and purification of chromosomeless minicells[9] of *E. coli* strain χ1488M and for labelling and analysis of protein in acrylamide-SDS gels have been described[7,23,24]. The sections of the gels (shown in Fig. 1) containing the appropriate bands were excised, digested with H_2O_2, treated with catalase, and counted[25]. The counts per minute of peptides of interest were internally normalised to either the β-lactamase band (expt 1) or the R49-amber nonsense repressor band (expt 2) by multiplying the observed counts by the ratio of counts in the internal control bands (normalisation ratio). t = truncated transposase peptide. R67 is another previously described amber nonsense mutation in the Tn*3* repressor gene[7].

* Parentheses indicate control c.p.m. determined for a slice of control gel removed from the same location as the band being analysed.

† Normalised counts.

Table 2 β-Galactosidase synthesis by *Tnp-lac* fusions plasmids

lac fusion plasmid	Plasmid *in trans*	β-Galactosidase (units)	Repression ratio
(1) pMC1103	—	5,300	
(2) pMC1103	pACYC999	1,100	
(3) pMC1103	pACYC999 R49	5,700	5.2 [pACYC999 R49/pACYC999]
(4) pMC1103	λsupF	3,000	1.8 [-/λsupF]
(5) pMC1082	—	130	
(6) pMC934	—	1,200	
(7) pMC934	ColE1	1,000	8.5 [ColE1/pACYC999]
(8) pMC934	pACYC999	120	
(9) pMC995	—	500	
(10) pMC995	ColE1	320	5.0 [ColE1/pACYC999]
(11) pMC995	pACYC999	66	

The structure of most of the pMC series of plasmids is shown in Fig. 2. pMC995 is structurally identical to pMC1082 except that its replicon is pACYC177 (ref. 26) rather than pSC101 (see text). The difference in copy number of the two vectors presumably is responsible for the observed difference in level of β-galactosidase (lines 5 and 9). The pACYC999 plasmid, the R49 amber mutation, the pACYC140 plasmid and the introduction of the λ supF amber suppressor have been described previously[7,26,27]. For β-galactosidase assays, strain MC1050 carrying the plasmids indicated was grown to appropriate cell density in minimal medium at 30 °C and assayed for the enzyme as described elsewhere[12].

lac gene fusions to isolate promoter-containing fragments of Tn*3* that are repressible by a wild-type Tn*3* *in trans*. Since the frequency of transposition and the synthesis of *Tnp* gene product are both regulated by the Tn*3* repressor (refs 5–7 and above), we hypothesised that at least some of the fragments isolated in this way might contain the *Tnp* gene promoter.

These experiments utilised the pMC874 plasmid (Fig. 3), which includes a gene encoding resistance to kanamycin (Km) plus a *lac* gene fragment in which the regulatory region for the *lacZ* gene and the codons for eight amino acids at the amino-terminal end of this gene have been removed and a *Bam*HI cleavage site has been introduced. The segment of *lacZ* that remains determines sufficient primary structure to yield a functional β-galactosidase enzyme, which can be expressed when a DNA fragment carrying both transcriptional and translational start signals is introduced into the *Bam*HI site[11]. In such a case, *lac* gene expression is placed under the control of a promoter, ribosomal binding site, and translational start codon on the inserted fragment and the resulting fusion peptide contains an amino-terminal end encoded by DNA sequences on the fragment.

A *Bam*HI endonuclease-generated fragment (931 *lac*) containing the *lac* gene segment derived from the pMC874 plasmid was introduced into the *BclI*$_b$ site of Tn*3* on pMC1097 as shown in Fig. 2 to yield the pMC1082 plasmid. Since the *Bam*HI and *BclI* endonucleases generate identical 4 base pair-long cohesive termini (that is, GATC), the 931 *lac* fragment can be readily inserted at either of the *BclI* sites identified within the *Tnp* gene or can be substituted for the Tn*3* DNA fragment between *BclI* sites a and b. The resulting constructs bring synthesis of the *lac* gene product under the control of regulatory signals for *Tnp*; β-galactosidase level can then be used as a direct indicator of *Tnp* expression.

As seen in Table 2, β-galactosidase is made by the pMC1082 Tn*3*–*lac* fusion plasmid, indicating that translation (as well as transcription, as noted above) of the *Tnp* gene commences at a location to the right of the *BclI*$_b$ site. A Lac$^+$ phenotype was observed with this plasmid and also with pMC1141, which contains an insertion of the 931 *lac* fusion fragment into the *BclI*$_b$ site of pMC1097 (Fig. 2). Since the reading frame of the DNA segments on both sides of the *Bam*HI/*BclI*$_b$ junction must be the same to yield expression of *lac* in the 931 *lac* fusion, and since the translational reading frame of the *lacZ* gene segment in the fusion is known in relation to its *Bam*HI cleavage site (see Fig. 3) and the *BclI*$_b$ site in the *Tnp* gene (Fig. 4), these results establish directly the reading frame for the *Tnp* gene. It is of some interest that *lac* expression from the pMC1082 fusion plasmid, which utilises both the transcriptional and translational signals of the *Tnp* gene, is less than 3% of the level of *lac* expression from pMC1103—in which the *Tnp* transcriptional promoter is linked to translational control signals of *lac*. This implies that relatively ineffective translation of a messenger RNA species transcribed from an efficient *Tnp* gene promoter is responsible for the observed weak expression of the *lacZ* gene in pMC1082. This interpretation is supported by results that show a markedly increased level of the *Tnp* gene product as a result of mutation within the putative translational control region for the gene (M.J.C., J.C. and S.N.C., unpublished data).

To localise more precisely the transcriptional and translational control region for the *Tnp* gene, we used the pMC874 plasmid to clone and isolate a small fragment of Tn*3* that could initiate Tn*3* -repressible expression of the *lac* gene segment. A plasmid that includes a wild-type Tn*3* element (such as pACYC999, which is ColE1 :: Tn*3*) was cleaved into small pieces using the *Sau*3A endonuclease, which recognises a 4-base pair sequence (GATC) that is a subset of the *Bam*HI recognition sequence[13], and which generates projecting ends identical to those produced by *Bam*HI. *Sau*3A-cleaved pACYC999 plasmid DNA was ligated to the *Bam*HI-digested pMC874 plasmid, and the composite molecules were introduced by transformation into *Escherichia coli* strain MC1050 which carries a *lac* deletion. One per cent of the transformants (transformation frequency 10^5 per μg DNA) showed a Lac$^+$ phenotype on lactose–MacConkey plates containing Km (10 μg ml^{-1}), suggesting that a *Sau*3A-generated DNA fragment(s) carrying transcriptional and translational start signals had been cloned in the appropriate orientation at the *Bam*HI site of pMC874.

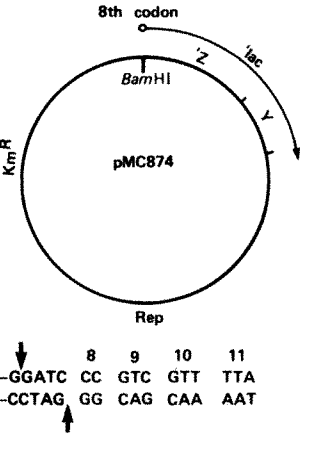


Fig. 3 Simplified map of pMC874 plasmid[11] indicating the position and orientation of the *lacZY* genes and the location of Km^r determinant and replication region (rep). This derivative of the pACYC177 plasmid[26] contains a unique *Bam*HI site, which results in cleavage before the eighth codon of the *lacZ* gene. DNA fragments that contain transcriptional signals and translational start codons yield a Lac$^+$ phenotype when introduced in frame with the *lacZ* gene at the *Bam*HI cleavage site. Addition of exogenously derived amino acids to the β-galactosidase protein does not affect its activity.

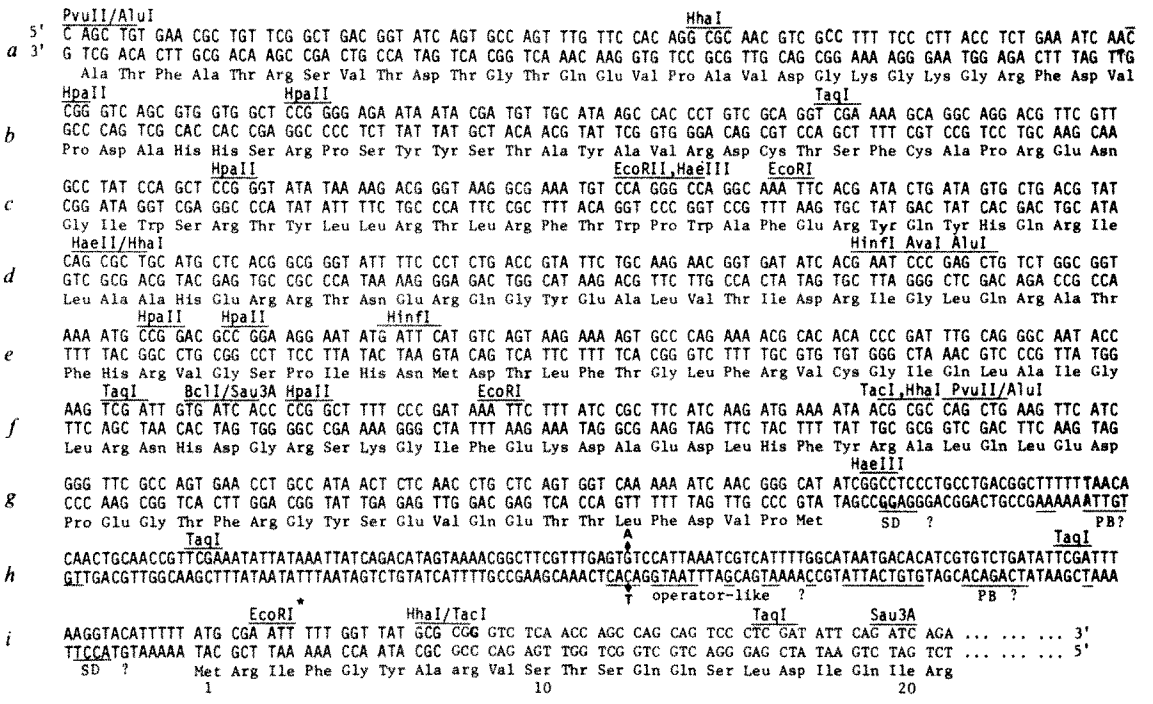


Fig. 4 Partial DNA sequence of the transposase and repressor genes of Tn*3*. The location of the Cis_{10} operator-constitutive mutation (which results in a C → T base change in the DNA strand corresponding to mRNA for the repressor gene)[7] is shown in the operator-like palindromic sequence in panel *h*. Putative Pribnow box[17] (PB) and Shine–Dalgarno[16] (SD) sequences are shown for both the repressor and transposase genes. The translational start site for the repressor protein has previously been shown to be at the methionine codon-labelled amino acid 1 in panel *i*. The translational start site for the transposase gene, which is read in an opposite direction from that of the repressor, is assigned to the methionine codon (labelled as amino acid 1) in panel *g*. It is possible, however, that initiation of the Tnp peptide occurs at another nearby location, as discussed in the text. The positions of some endonuclease cleavage sites are indicated. *Sau*3A fragment VII of Tn*3* is a 359-base pair fragment extending from the *BclI*/*Sau*3A site at the 46th amino acid of the transposase gene (panel *f*) to the 20th amino acid of the repressor gene (panel *i*). The DNA sequence was determined by the Maxam and Gilbert procedure[31] by analysis of multiple fragments within the region shown, and sequences were confirmed from both strands of the duplex DNA. Details of which specific endonuclease-generated fragments were sequenced and the primary sequencing data are available from the authors on request. All fragments were labelled with [γ-^{32}P] ATP (~3,000 Ci mmol^{-1}, ICN) at the 5′ends using bacteriophage T4 polynucleotide kinase.

Twenty-five randomly selected Lac$^+$ colonies were purified by restreaking and examined for the ability of the *lac* gene carried by pMC874 derivatives to be repressed *in trans* by a wild-type Tn*3*; four of these colonies showed repression of Lac$^+$ phenotype *in trans* by a wild-type Tn*3* element carried by either bacteriophage λ or the pSC101 plasmid. The restriction endonuclease cleavage pattern of plasmid DNA from the four repressible Lac$^+$ isolates was compared by electrophoresis in 5% polyacrylamide gels with the pattern seen for the pMC874, ColEl and pACYC999 (ColEl::Tn*3*) plasmids (data not shown). The only cloned fragment detectable in one of the plasmids (pMC934) was a *Sau*3A fragment similar in size to one included within Tn*3*; the other three Lac$^+$ isolates contained plasmids that included this fragment plus additional *Sau*3A fragments derived from Tn*3* or ColEl.

Endonuclease mapping (unpublished data) of the *Sau*3A fragment common to the four repressible Tn*3*–*lac* fusion plasmids (*Sau*3A fragment VII of pACYC999) showed that it was the same Tn*3* fragment that contains the coding sequence for the NH_2-terminal end of the repressor gene and the regulatory region for the gene (Fig. 4; see also ref. 7). The orientation of insertion of the *Sau*3A-generated fragment in two of these clones (including that carrying the pMC934 plasmid) was found to juxtapose the regulatory sequences for the repressor and the β-galactosidase gene of pMC874. Moreover, a nucleotide sequence of undetermined origin and too short to be detected on 5% polyacrylamide gels was present at the *Sau*3A/*Bam*HI junction of pMC934, bringing the translational reading frames of the repressor and β-galactosidase segments of the fused protein into concordance. This finding suggested that the extraneous fragments identified in the other repressible Lac$^+$ isolates might also be accomplishing a frame shift that places *lac* in the same translational reading frame as the repressor.

*Sau*3A fragment VII was purified from pACYC999 plasmid DNA by electrophoresis on 5% polyacrylamide gel and ligated to *Bam*HI-cleaved pMC874 DNA in the absence of detectable extraneous DNA fragments that might shift the translational reading frame. Plasmid DNA from four randomly selected Lac$^+$ repressible clones was examined by gel electrophoresis and in each case was found to contain *Sau*3A fragment VII in an orientation opposite to the orientation observed for pMC934. The plasmid present in one of these clones was designated pMC995. β-Galactosidase encoded by both the pMC934 and the pMC995 plasmids was repressed by a wild-type Tn*3* *in trans* (Table 2, lines 6–11) showing that transcription and translation proceed from *Sau*3A fragment VII in divergent directions and that the divergent promoters are both regulated by the repressor gene product. These findings provide direct evidence for bidirectionality of the genetic unit containing *Tnp* and repressor genes, which was first inferred from polyacrylamide gels of gene products made in minicells. Although other promoter regions within the pACYC999 plasmid have been detected by the use of pMC874 plasmid translational fusions, the two promoters we have identified on the *Sau*3A fragment VII of Tn*3* were the only ones found to be regulated by the repressor.

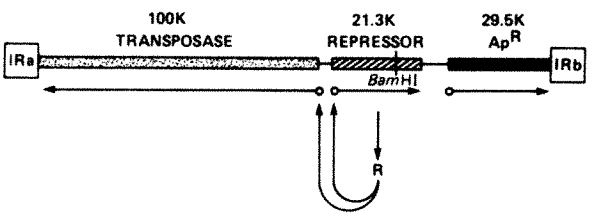


Fig. 5 Schematic diagram showing locations of Tn*3* genes and their regulatory signals. As shown, a 21.3K repressor peptide acts to reduce transcription from the promotors for the transposase and repressor genes, which are bidirectionally expressed.

Nucleotide sequence analysis of the transcriptional and translational control region of the transposase gene

Nucleotide sequence analysis was carried out for the Tn*3* DNA segment encoding the NH_2-terminal portion of the *Tnp* gene product and the regulatory region for this gene (Fig. 4). As indicated above, the 359 base-pair long *Sau*3A fragment VII of the pACYC999 plasmid contains the transcriptional and translational control region, plus the NH_2-terminal sequences for both the repressor and transposase peptides; together these genes appear to constitute a bidirectional genetic unit that regulates transposition of Tn*3*. Amino acids for the Tnp peptide are assigned from the ATG (AUG) codon located to the left of the single *Hae*III site within the intergenic region; no termination codon in the same frame as this ATG was seen throughout the DNA segment we have sequenced (585 base pairs). The presence on the coding strand of a GGAGG putative Shine–Dalgarno ribosomal binding sequence[16] preceding the ATG codon, and a possible Pribnow box[17] for binding of RNA polymerase[18] several nucleotides further towards the 5′ end of the coding strand, is consistent with this assignment. However, another possibility is that initiation of the Tnp peptide occurs at the GTG (GUG) codon that is 33 nucleotides nearer to the 5′ end of the coding strand. This codon, which is known to be capable of initiation of translation[19], is also in the correct reading frame as ascertained by our DNA sequence analysis.

The occurrence, mid-way between the transcriptional start signals for the repressor and *Tnp* gene, of the palindromic sequence proposed by us to be the operator structure[20] for the repressor gene of Tn*3*, raises the possibility that this single operator may control both components of the bidirectional Tn*3* transposition unit. Control of two promoters by a single operator region located between divergent genes has been reported previously for the bacteriophage λ*cI–cro* operon[21].

Overall regulation of the Tn*3* transposition unit

The present results, and those reported earlier[7], provide a structural and functional basis for regulation of the transposition frequency of Tn*3* and also explain the observation that structural integrity of the region to the left of the *Bam*HI site of Tn*3* is required for transposition to occur[2,3,8]. The self-regulated repressor gene is shown here to be part of a repressor-controlled divergent genetic unit (Fig. 5) that includes a gene encoding a large peptide essential for transposition of Tn*3*.

Transposition is thought to be a multi-step process that involves DNA nicking and ligation steps, plus replication-related functions[22]. The large size of the Tnp peptide may reflect an ability to carry out multiple steps in the transposition process.

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Structure and sequence of the multihaem cytochrome c_3

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The molecular structure of cytochrome c_3 isolated from Desulfovibrio desulfuricans *has been solved on the basis of its crystallographic determination at 2.5 Å resolution and of an essentially complete sequence. The molecule consists of a single polypeptide chain wrapped around a very compact core of four non-parallel haems which present a relatively high degree of exposure to the solvent. Alignment of the amino acid sequences of cytochrome c_3 from several species suggests that the structure reported here is characteristic of the cytochrome c_3 group.*

SULPHATE reducing bacteria of the genus *Desulfovibrio* are characterised by the presence of a specific electron carrier protein which has been called cytochrome c_3 (ref. 1). These proteins have recently been defined as a unique class of haemoproteins which contain four haems per molecular weight of about 13,000, are autoxidisable and bear a negative oxidoreduction potential[2].

With the publication of the amino acid sequences of cytochromes c_3 from three different species of *Desulfovibrio*[3–5], it became clear that in spite of large differences in their amino acid composition cytochromes c_3 constitute an homologous class of proteins characterised by the presence of four haem attachment

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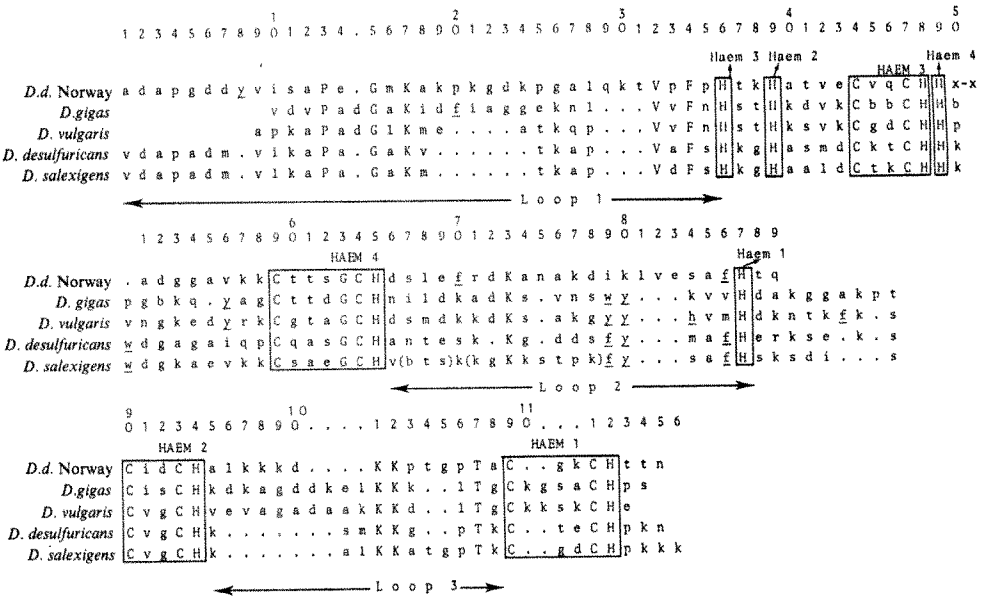


Fig. 1 Sequence homology of cytochromes c_3. The sequences are expressed in the one letter code[27]; *D.d.* Norway is the reference sequence; capitals indicate residues common to each sequence. Haem attachment sites are enclosed in boxes and other aromatic residues are underlined.

sites, that is, -Cys-x-x(x-x)-Cys-His-. The sequence of the cytochrome c_3 from *Desulfovibrio desulfuricans* Strain Norway (*D.d.* Norway) is almost completed and is used in the present three-dimensional study (Fig. 1).

The structural organisation of four porphyrin rings within a relatively short polypeptide chain should be quite remarkable and already extensive EPR[6], NMR[7,8] and Mossbauer[9] studies have been undertaken and led to some important results: (1) the sixth axial haem ligand is a nitrogen atom of a histidine residue, therefore each iron coordination complex in cytochrome c_3 is of bis-histidinyl type; (2) close haem–haem intramolecular interactions are strongly suggested in view of short inter-iron distances (~10 Å); (3) the slow electron exchange observed between haems favours the proposal that these proteins do not act as simple electron conductors but more likely as storage devices for electrons; (4) an outline structure of *D. vulgaris* cytochrome c_3 based on sequence, EPR and NMR studies has been proposed by Dobson *et al.*[8] and will be discussed later.

The physiological electron donor and/or acceptor for cytochrome c_3 is hydrogenase. In *Desulfovibrio* species the presence of cytochrome c_3 is a requirement for the electron transfer to occur in contrast to *Clostridium* species in which this process takes place by direct coupling between hydrogenase and low redox potential proteins such as ferredoxin, flavodoxin and rubredoxin[10]. Insofar as the physiological properties of the *D.d.* Norway cytochrome c_3 are known they deserve a few comments since they differ remarkably from those of cytochrome of *D. gigas* and *D. vulgaris*. In the Norway strain cytochrome c_3 has a sulphur reductase activity[11]. Cytochrome c_3 from *D. vulgaris* also reduces sulphur but its activity is strongly inhibited by hydrogen sulphide, the product of the reaction. Another property of *D.d.* Norway cytochrome c_3 also favours a change in the function of this protein: it couples the reduction of thiosulphate from molecular hydrogen in cell-free extracts whereas *D. gigas* c_3 does not. In this latter organism this function is the property of another cytochrome absent in the Norway strain, containing eight haems per molecular weight of 26,000 and also named c_3 (ref. 12). It is noteworthy that cytochrome c_3 is not the only *c*-type cytochrome in *D.d.* Norway since a monohaem cytochrome $c_{553(550)}$ has recently been purified[13].

The X-ray crystal structures of several monohaem *c*-type cytochromes led to the important conclusions that these molecules appear to have a similar 'cytochrome fold' and to belong to the same evolutionary group, indicating a common origin for electron transport chains in photosynthesis and respiration[14–16]. We present here the first high resolution structure of a multihaem cytochrome in which the complete polypeptide chain around a compact four haem arrangement could be traced throughout.

Experimental methods

Cytochrome c_3 from *D.d.* Norway was crystallised in 50% 2-methyl-2,4-pentanediol at room temperature and at *p*H 7.7. Rod-shaped crystals were obtained in trigonal space group $P3_1$ ($P3_2$) with unit cell parameters $a = b = 43.72$ Å $c = 64.45$ Å (ref. 17).

Full details of the structural analysis will be reported elsewhere but the strategy may briefly be outlined here. Three mercury derivatives were used, all prepared by soaking native crystals in appropriate solutions ($HgCl_2$, $Hg(SCN)_2$, ethylmercuriphosphate). Replicated data including Bijvoet reflections were collected on a CAD-4 Enraf-Nonius diffractometer with Ni-filtered CuK α radiation and an ω-scan procedure. The single site (A) of derivative $HgCl_2$ was found to be common to the three derivatives. Heavy atom parameters were refined by the F_{HLE} procedure of Dodson and Vijayan[18], with empirically determined values of the real/imaginary scattering ratio[19]. Before, the final phase calculation combining isomorphous and anomalous measurements, accurate haem-iron coordinates were required, as the anomalous scattering from native iron atoms is far from being negligible. Native protein phases were first calculated from isomorphous data alone[20]. The mean figure of merit for the 4,400 reflections at 2.5 Å resolution was relatively low; 0.66. At this point the electron-density map allowed a straightforward identification of the molecular boundary and of four regions of significantly higher density which obviously could be attributed to the four haem groups of the molecule. Moreover the iron atom parameters could be refined properly by a least-squares procedure using the observed native anomalous differences, in a way similar to that described for cytochrome b_5 (ref. 21). With accurate iron coordinates it was then possible to calculate a second set of 'best' phases based on both isomorphous and anomalous contributions. This led to a mean figure of merit of 0.76 falling off from 0.82 at 6 Å resolution to 0.64 at 2.5 Å resolution. The high quality of the electron-density map calculated with these improved phases can be judged from Fig. 2.

Description of the molecule

The present model is based on the polypeptide chain continuity observed on the electron density map drawn at the scale of 0.4 Å cm^{-1} and on sequence information. From this 'mini' map the high contrast between protein and solvent regions allows an easy separation between molecules. Cytochrome c_3 appears as a

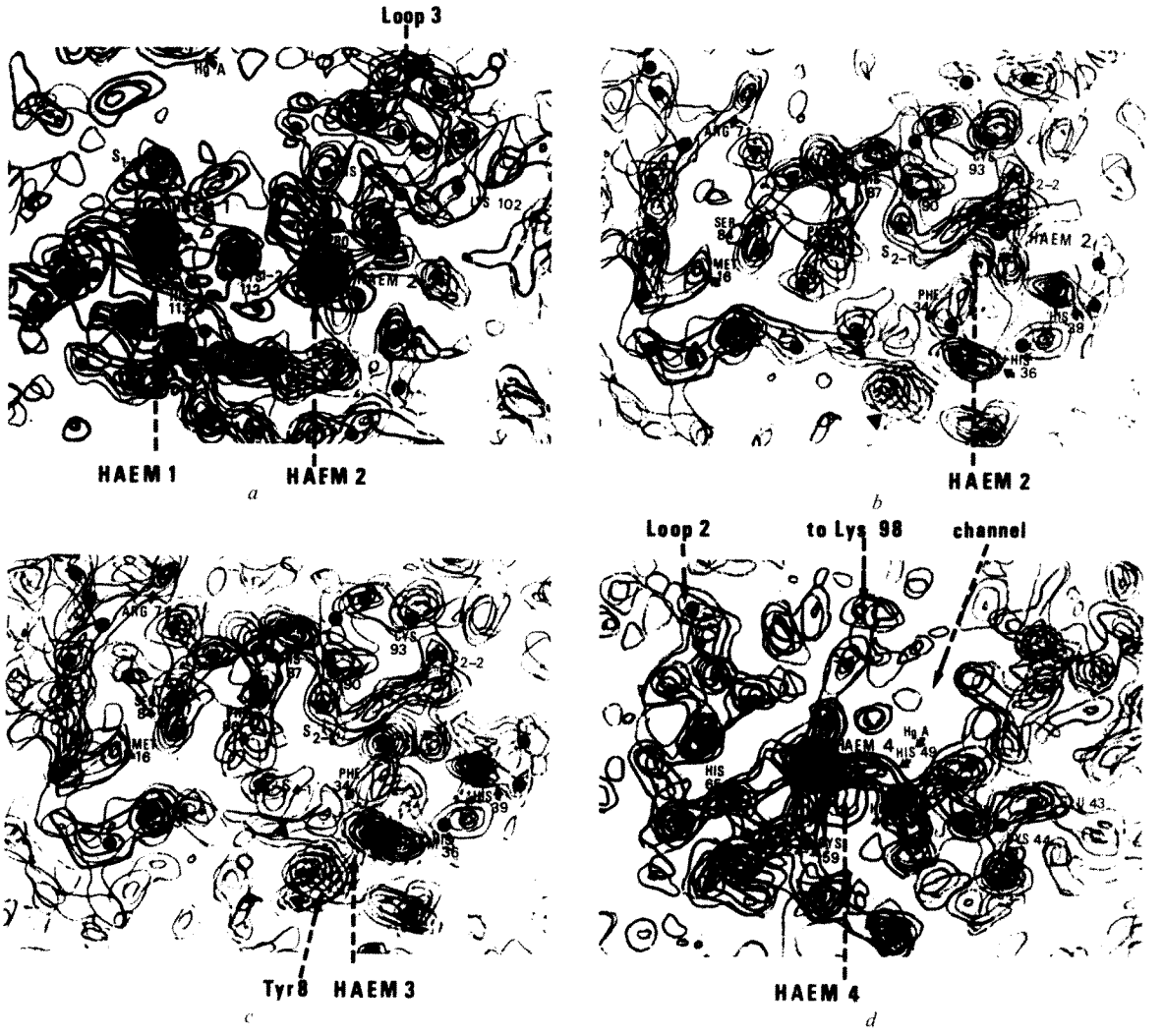


Fig. 2 The electron density map in the different haem regions. The sections are perpendicular to the three-fold axis. *a*, Haem 1 and the top of haem 2. Note the propionates of haem 2 and the loop 3; *b*, Haem 2 with His 39, Cys 90 and Cys 93; *c*, Haem 3 nearly parallel to the plane of the section. Tyr 8 is hydrogen bonded to one of its propionic acid groups; *d*, Haem 4 and the two consecutive His 48 and His 49. ○, Haem centre; ★, sulphur atom of cystein residue; ●, α-carbon; ►, carboxylate of propionic acid.

bean-shaped molecule of overall dimensions 45 × 28 × 32 Å the last figure corresponding to the three-fold axis direction. The course of the main chain could be traced entirely and the orientation of the four porphyrin rings as well as the haem binding groups are clearly visible.

The folding of the α-carbon backbone is shown diagrammatically in Figs. 3 and 4 together with the haem arrangement. Due to lack of overlap information a very small part of the sequence after His 49 has not yet been identified. Indeed the electron-density map clearly indicates the presence of one or two residues between His 49 and the next Ala and we decided to include one amino acid presently not identified and labelled X–X in position 50. A definite answer must await the final sequence data and the optimum fitting of the molecule in the Richards box. Many side chains are already identified on the mini map (Fig. 2), and are in excellent agreement with the sequence. In brief the chain consists (Figs 3 and 4) of three external loops and interconnecting haem segments, and adopts a right-handed helical conformation around residues 86–100. This helical segment runs roughly parallel to the porphyrin plane of haem 1 and contains two histidines, at residues 87 and 94, respectively linked to haems 1 and 2. At the junction of loops 2 and 3 the molecule is bent into a V-form giving rise to a deep depression. Both *N* and *C* terminals are clearly visible and lie on the surface of the molecule. Most of the hydrophilic amino acids are located on these external loops. Among the 17 lysines and the 10 aspartic acids of the molecule, 13 and 8 respectively are found on these loops. For instance loop 3 contains the remarkable segment Lys 97-Lys-Asp-Lys-Lys 102.

Haem core

The most remarkable feature of the c_3 structure is the compact organisation of the four haems within the molecule. These lie near the surface of the protein, haems 2 and 4 sharing the same long groove dividing the molecule in two halves with one haem moiety in each. The short iron-to-iron distances which range from 10.9 Å (Fe2–Fe3) to 17.3 Å (Fe1–Fe3) reflect the close packing of the four non-parallel porphyrin rings as indicated on Figs. 3 and 4. Haems 1 and 3 are nearly parallel and roughly perpendicular to porphyrin rings 2 and 4, a spatial arrangement which does not reveal any internal symmetry.

The electron density in the neighbourhood of the four redox centres is shown on Fig. 2 where some essential features can be observed. First of all, each haem is linked, as in cytochrome *c*, to the apoprotein by two thioether bonds involving well resolved cysteines. The fifth and sixth ligands of each iron atom are provided by histidyl residues, as in cytochrome b_5. This is in agreement with previous NMR studies[7,8], ruling out Met and Lys as possible axial haem ligands. Indeed the sole methionine (Met 16) clearly visible on Fig. 2*b* and *c* is definitely not bound

Table 1 Residues close to the various haems

Haem 1	Haem 2	Haem 3	Haem 4
Val 32	Val 32, 42	Val 42, 56	x–x 50
Leu 28	Gly 105	Ala 3	Ala 85
Ala 27	Pro 33, 35	Pro 4	Gln 89
Phe 86	Pro 103, 106	His 39	Phe 86
Thr 31, 107	Phe 34	Phe 34	Lys 57, 80
Ser 84	Thr 104	Tyr 8	
	Lys 38, 97	Lys 58	

to any haem. The two consecutive histidines (His 48, 49), a highly conserved feature of all known c_3 sequences, connect directly the iron atoms 3 and 4 which correspond to the less and the most accessible haems respectively. It is to be noted that each haem group is peculiar in terms of environment and solvent exposure. Residues in the vicinity of the different redox centres are indicated in Table 1. The propionic groups located on the electron density map reflect also the various haem exposures. For haems 2 and 4, which lie in the groove largely open to the external medium, the propionates extend to the solvent whereas for haem 3 they point to the interior of the molecule: one forms a hydrogen bond with the well resolved Tyr 8 (Fig. 2*c*) and the second is also probably hydrogen bonded to Lys 58. Haem 1 seems to present an intermediary situation as it is partly covered by loop 1 and the *C*-terminal region. One of its propionic acids points towards loop 1 and is probably hydrogen bonded whereas the second appears free in the solvent. At this point some interesting intermolecular contacts are observed. One of the propionic groups of haem 4 appears to be hydrogen bonded to Lys 98 of a neighbouring molecule. This haem also faces a channel of about 7 Å diameter running throughout the crystal and providing easy access to His 49, the common binding site (A) for the three mercury derivatives (Fig. 2*d*).

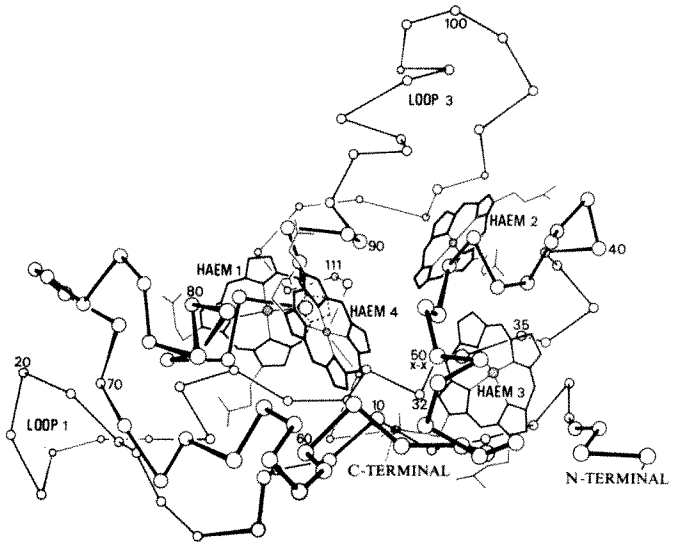


Fig. 3 A drawing of the main polypeptide chain and of the haem arrangement in cytochrome c_3. Haem numbering is arbitrary: when looking along the three-fold axis one goes from haem 1 to haem 4 which are lying respectively near the bottom and the top of the molecule. The view is from the three-fold axis which is perpendicular to the plane of the paper. The α-carbon positions were derived from the mini map and are represented by circles whose sizes indicate their relative depth. Coordinates of haem atoms and haem ligands were measured from a Kendrew wire model built in a Richards box. Inter-iron distances are: Fe1–Fe2 = 12.7 Å; Fe1–Fe3 = 17.3 Å; Fe1–Fe4 = 16.3 Å; Fe2–Fe3 = 10.9 Å; Fe2–Fe4 = 16.8 Å; Fe3–Fe4 = 12.8 Å.

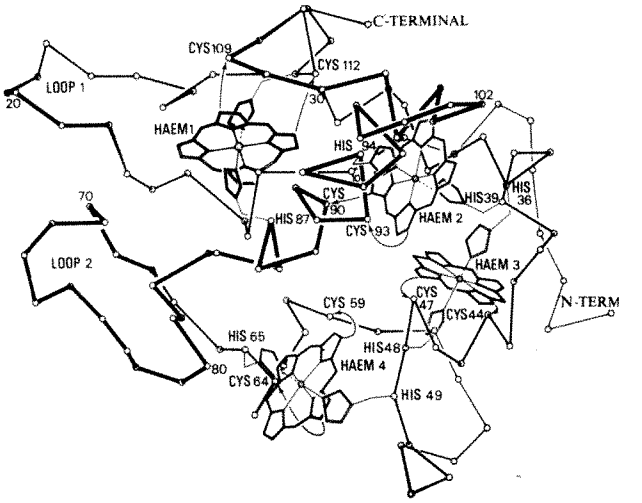


Fig. 4 The main polypeptide chain and the haem arrangement, the direction of view being approximately parallel to the a axis. Angles between porphyrin rings are 85° for haems 3–2, 80° for haems 3–4, 90° for haems 1–2, and 60° for haems 1–4. The shortest inter-haem distances (~5 Å) involving pyrrole rings are found between haems 1 and 2 and haems 2 and 3.

A detailed description of the haem environments, which should help to elucidate the intriguing problem of the haem-haem interactions, will be deferred until the completion of the model (2 cm $Å^{-1}$).

Discussion

The cytochromes c_3 of various species present quite different biochemical behaviours which could imply some changes in their three-dimensional structure, so the question arises as to whether the structure of *D.d.* Norway cytochrome c_3 reflects that of the other cytochromes c_3. Comparison of primary structure of several cytochromes c_3 shows a relatively low degree of homology: among the five available sequences only 25% of the total residues remain invariant, such a variability being uncommon in the *c*-type cytochrome family. A possible alignment of these sequences is proposed in Fig. 1 with the *D.d.* Norway structure as reference. This alignment is based on the haem binding groups, that is the four invariant Cys—Cys-His and the four invariant histidyl residues. It is clear that most of the changes appear in the external loops and could be accommodated without disturbing the haem core. However an important modification concerns the region between His 87 and Cys 90. Examination of the reference structure suggests that this short (two amino acids) chain in *D.d.* Norway can be replaced by a longer (six to nine amino acids) external loop, and such an insertion does not necessarily imply a change in the orientation of the proximate haem 2. At present there are no structural arguments to support a different arrangement of the haem core in the cytochromes c_3.

More subtle differences must be responsible for differentiated reactivities of cytochromes c_3, for instance the high variability of aromatic side chains which is not encountered in the cytochrome *c* family. Striking evidences are shown (Fig. 1). Tyr 8 which is hydrogen bonded to the propionic group of haem 3 is specific for *D.d.* Norway which in contrast does not contain the tyrosines and tryptophans found in the other cytochromes in loop 2 or between haems 3 and 4. Phe 34 which is located between haems 2 and 3 in the *D.d.* Norway structure (Figs. 2*b* and *c*) is invariant in the five molecules suggesting that this residue is probably important for the electron transfer mechanism. On the other hand Phe 86, between haems 1 and 4 (Fig. 2*c*), is only found in two cytochromes. Extra aromatic residues are underlined in Fig. 1.

Despite these local variations it is likely that the three-dimensional structure reported here is representative of the

cytochrome c_3 molecule generally. In this respect the model proposed by Dobson *et al.* for *D. vulgaris* is in conflict with the actual structure. There are at least two major differences. First, the short chain segments between haems 2 and 3 and between haems 3 and 4 do not require the parallelism between the porphyrin rings but, on the contrary, haem 3 is roughly perpendicular to haem 2 and haem 4. Second, the folding suggested previously by these authors was based on the idea that the polypeptide chain is wrapped around an inner haem region. This is not true in our model with regard to the external loops and the exposure of the haem moieties as described above. In fact it would be interesting to refine the interpretation of the NMR and EPR data on the basis of our three-dimensional model. Future work should also elucidate the dependence of the redox potential on the exposure of each haem as already established for the cytochrome *c* family[22]. It is likely that such a correlation will hold for the cytochromes c_3. As mentioned above each haem obviously has a different exposure to solvent and consequently should present a distinct reduction potential. Indeed several midpoint potentials have been measured either by EPR (ref. 6) or by differential pulse polarography[23] for different cytochromes c_3. The lowest value $E_0' = -340$ mV for *D.d.* Norway should be assigned to the most accessible haems in the structure, namely haem 2 or 4. Such low potentials are not detected in the three-haem cytochrome c_7 from the sulphur-reducing anaerobic bacteria *Desulforomonas acetoxidans*, $E_0' \simeq -200$ mV. It is noteworthy that sequence comparisons show that this cytochrome c_7 can be derived from the c_3 molecule by deletion of a peptide including the binding sites of haem 4. In order to correlate these observations it will be interesting to know the three-dimensional structure of cytochrome c_7 which is now being studied in our laboratory[24].

Recently the interest in a detailed study of cytochrome c_3 from *D.d.* Norway has increased as it has been demonstrated that this protein acts as a very efficient electron mediator in accepting electrons from chloroplasts for the photoproduction of hydrogen from water[25]. In this connection the cluster of four cytochromes *c* described as the reaction centre at the membranes of the red photosynthetic bacteria *Chromatium vinosum*[26] shows an intriguing similarity with the haem core of cytochrome c_3. It is tempting to speculate that such a close proximity between the haems (8–10 Å in *C. vinosum*, as in cytochrome c_3) is an essential arrangement for electron exchange between chloroplast membrane, cytochrome c_3 and hydrogenase.

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letters

The IR energy distribution of SS433

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SS433 is an extraordinary emission-line object showing periodically varying velocities of up to 50,000 km s^{-1} (refs 1, 2). It is also a variable radio source[3,4] and an X-ray source[5]. Infrared observations of SS433 in the 1–4 μm range have been reported elsewhere[6–10]. The data that we present here include observations at 1–10 μm and indicate first that there are substantial infrared colour variations from night to night and second that there is a sharp long-wavelength cutoff in the IR energy distribution, which was not seen in the earlier, more limited data. The discovery of this cutoff allows the diameter and electron density of the emitting region to be estimated.

Photometric measurements were made on the nights of 29, 30 and 31 May 1979 UT, using the University of Hawaii 2.2-m telescope on Mauna Kea. The 1.2–4.8 μm observations employed an InSb detector and a 6 arc s diaphragm while the broadband 8–13 μm observations used a gallium-doped germanium bolometer and a 6 arc s diaphragm. On each night all the observations were completed within 20 min of the mean time given in Fig. 1, except that the 10 μm observation on the 29 May was made about 2.5 h earlier than the time quoted.

Figure 1 shows that between 1.2 and 4.8 μm substantial night-to-night variations exist with a suggestion that the variations are stronger at longer wavelengths. Unfortunately, no 10 μm data are available for 31 May, but a later observation by Becklin and Telesco, made during the commissioning of the 3.0-m NASA IR Telescope Facility on 1979 June 14.4 gave a 10 μm flux density of $\log S_\nu = -26.82 \pm 0.04$ a factor of 30% less than that observed 15 days earlier. At the same time C. Lonsdale and H. M. Dyck (personal communication) measured a 2.2-μm flux density of $\log S_\nu = -26.54 \pm 0.04$, a value lower than earlier measurements. The IR night-to-night variations of both flux density and colour therefore seem to be substantially larger than those reported in any other wavelength range.

The proposal by Giles *et al.*[9] that the IR emission from SS433 is due to reddened free–free emission from an ionised plasma provides a natural explanation for the shape of the energy

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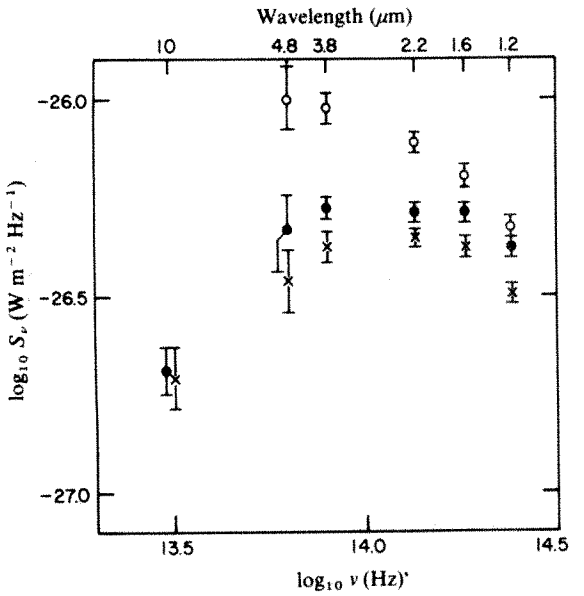


Fig. 1 The IR energy distribution of SS433 on: ●, 29.64 May 1979 UT; ×, 30.51 May 1979 UT; ○, 31.60 May 1979 UT.

distribution shown in Fig. 1, in that the decrease in flux density at wavelengths longward of 5 μm can be attributed to self-absorption. Although it is probable that the standard relationships for H II regions (see ref. 11) are inaccurate at the high column densities encountered in SS433, we may make estimates of the physical parameters of its emitting regions by assuming an electron temperature of 10^4 K and normal recombination theory. From the flux density (0.5 Jy) and the wavelength at the turnover frequency (5 μm) we can derive an angular diameter of order 2×10^{-4} arc s and an emission measure ($\int N_e^2\,dl$) of the order of 10^{17} pc cm^{-6}. If a distance of 3.5 kpc is assumed[1] we estimate a physical size for the emitting region of order 1 AU, an electron density of the order of 10^{11} cm^{-3} and a photoionisation rate of 10^{48} s^{-1}, a value that is similar to that of a late O type star. The size derived here is comfortably smaller than any upper limits implied by the observed variability of the source, while the electron density is high enough to account for the weakness of the forbidden lines in the optical spectrum[1]. The mass of ionised gas, assumed uniform density, is of the order of $10^{-7}M_\odot$.

If the IR radiation arises from free–free emission then a possible explanation for the fact that the variability is greater at longer wavelengths suggests itself. If an H II region is ionisation bounded its optically thin flux density is dependent on the rate of production of ionising photons, but almost independent of the geometry and electron density. At longer wavelengths, however, where self-absorption exists along at least some lines of sight the flux density is much more sensitive to changes in geometry, viewing angle and electron density. Further studies of the time variations of the IR energy distribution of SS433 may therefore help to determine the shape and the properties of its emitting region.

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Magnetic field of Jupiter and the volcanism and rotation of the Galilean satellites

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The discovery by Voyager 1 of several active volcanoes on Io[1,2] opens up a new approach to problems such as the nature and consequences of volcanism on the celestial bodies. In the past 50 yr only Vsekhsviatsky has actively considered modern volcanism on planets, in particular on Moon-like bodies[3,4]. He proceeded from the concept of eruptive origin of comets, however, and did not suggest a physical mechanism to explain the volcanic activity he had postulated. The absence of modern volcanism on the Moon and Mars was against Vsekhsviatsky's ideas and few people considered them seriously, especially in connection with the possible volcanic ejection of icy blocks. Progress was made by Peale *et al.*[5] who predicted the existence of the volcanoes just before they were discovered, and suggested a physical mechanism for the volcanism on Io. They suggested that the tidal dissipation caused by the orbit eccentricity could produce a power $W_{tid}\approx1.6\times10^{12}$ W in Io and 8×10^{10} W in Europa. If the satellites are nonhomogeneous and have a molten interior, the energy release can increase several times. Peal *et al.* estimated the thickness of Io's solid crust as only 18 km. However, "how can Io's south polar region support what appear to be towering mountains? (ref. 6)". (The typical relief is ~10km here[2]). Also, the lack of a detectable intrinsic magnetic field of Io[8] does not favour a fully molten interior. I have previously pointed out that Io's and Europa's orbiting in the strong magnetic field of Jupiter may cause their volcanic activity[7] and this can be confirmed by simple calculations[8,9]. Here I shall estimate the energetics in more detail and discuss some peculiarities of the volcanism itself and consequences of the electromagnetic action mainly on the rocky satellites (the 'icy' volcanism will be considered elsewhere).

Most of the outer planets' satellites are located in the planetary magnetospheres. Io is unique: its interaction with the magnetosphere leads to radiation of ~10^8 W in the decametric band[10]. The radiation is believed to be caused by unipolar generation of the electric current due to Io's movement across the jovian magnetic field[10–12]. The current flows from Io in Jupiter's plasmasphere along the magnetic force tubes down to the highest, weakly conducting ionospheric layers of the jovian atmosphere, just where the radiation is generated. The energy released by Io's interaction with Jupiter's field, has been predicted as, or even more than, 10^{15}–10^{16} W but from estimations of the upper limit of Io's secular acceleration it seems that at present it cannot exceed 10^{14} W (see ref. 10). A more moderate value follows from the consideration that a current generated by the movement of a body across a magnetic field will hardly exceed the current $I_{max}=2\pi BR_s/\mu_0$, whose own magnetic field is equal to the initial field[13].

Table 1 presents the satellites' parameters: D, their distances to the centre of Jupiter; R_s, their radii; M_s, their masses; B, the magnetic field; $\Delta U=2R_sv_{rel}B$, the generated potential difference; I_{max}; $W_{max}=\Delta UI_{max}$ and power W_{rad} supplied by radioactive fission on the assumption of the chondritic composition (5×10^{-12} W kg^{-1}); the rocky cores of Ganymede and Callisto are assumed to be ~1/3 of their mass. For Callisto $W_{max}\ll W_{rad}$, for Ganymede $W_{max}\lesssim W_{rad}$, for Europa $W_{max}\gtrsim W_{rad}$, but for Io and Amalthea $W_{max}\gg W_{rad}$. Note also that $W_{max}>W_{tid}$, although they have the same order of magnitude. The relation between W_{max} and W_{rad} suggest the existence of highly active volcanism on Io and, probably, on Europa.

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Table 1 Magnetic data for Jupiter's satellites

Satellite	D (10^5 km)	R_s (km)	M_s (10^{23} kg)	B (G)	ΔU (kV)	I_{max} (10^6 A)	W_{max} (10^{12} W)	W_{rad} (10^{12} W)	Δt (yr)	ΔP(Myr) (at $P_0 = 10$h)
Amalthea	1.81	130; 70	0.0002	0.26	35	9	0.31	0.0001	2.7×10^8	
Io	4.22	1820	0.891	0.020	414	18	7.45	0.45	3.6×10^{11}	0.186
Europa	6.71	1560	0.487	0.0051	159	3.9	0.62	0.24	3.4×10^{12}	0.25
Ganymede	10.7	2640	1.490	0.00125	113	1.6	0.18	0.25	4.7×10^{13}	0.68
Callisto	18.8	2420	1.074	0.00023	35	0.3	0.01	0.18	8.9×10^{14}	1.07
Amalthio	1.81	1820	0.891	0.26	500	228.0	114.00	0.45	3.4×10^9	0.08

When estimating Io's radio-wave generation, most of the energy is believed to release in the jovian ionosphere as its resistance is larger compared with Io's integral resistance[12]. Apparently there has not been any estimation of the energy input in Io itself. Note, however, that all available estimations of any interaction parameters of Ioian and jovian field are ambiguous and unreliable[10]. The current values under consideration (restricted by the conductivity of the jovian atmosphere) greatly exceed 10^5 A (ref. 14): for instance, $I = 5\times10^6$ A (ref. 15), up to $I = I_{max}$ and even sometimes considerably higher than I_{max} ($I = 2.5\times10^8$ A follows from $W = 10^{14}$ W). Note that the current is limited by conductivity only at $I < I_{max}$. Also, even if the conductivity is large, I remains nearly equal to I_{max} (ref. 13). Thus, one can take $I \approx I_{max}$ as a characteristic value, which is confirmed by the direct measurement by Voyager[8] of the oppositely directed currents, flowing along one wing of Io-flux tube. The current was estimated as 4.8×10^6 A, hence the total current through Io's body may be as high as, or even more than, 9.6×10^6 A; thus it approaches the value of I_{max} (the currents in the opposite wings of the tube may not be equal and their strength may vary considerably with time[14]). The above choice is also based on the consideration that when the current flows through a gas producing an additional ionisation, and when it flows through solid dielectrics or semiconductors, heating them and ultimately causing thermal breakdown, the volt–current characteristics have a negative slope and the current stabilisation is achieved by other means such as a ballast resistance or, as in this case, by the condition $I \lesssim I_{max}$. Sometimes, an opinion is expressed about the small conductance of Io[16,17] based on the low conductivity of the outermost layers of the Moon with thickness $\lesssim 1$ km, and then it is concluded that the current closes not through Io itself, but through its ionosphere. Clearly, however, any tenuous ionosphere should be momentarily stripped off by the Ampere forces if the magnetic field slips freely relative to nonconducting satellite, but it may exist if the current penetrates the satellite and the field is frozen somehow in its body. Voyager observations[18] reveal numerous warm surface spots on Io, the temperature of which are 200–290 K and maybe even higher. These spots could be entrances for the electric current in the satellite's body through the thin outermost layer. Very crude estimations of the rocky satellite resistance can then be made using available data for the Moon[19]. (The conductivity σ of dirty ices from which the icy satellite crust is formed is higher compared with rocks, about 10^{-4} S, ref. 20). The main input in the resistance comes from the outer 175 km; here $\sigma = 10^{-6}$ S. Then the column with base 1 m^2 has resistance $\sim 2\times10^{11}\ \Omega$, and all the satellite body $\sim 2\times2\times10^{11}/\pi R_s^2\ \Omega$. It gives for Io, 0.04 Ω; for Europa, 0.06 Ω, which is close to values needed for $I = I_{max}$. Thus W_{max} is the right order of magnitude for the additional energy input in the satellite body. In fact, the resistance will be somewhat lower: in the heated satellites the thickness of the low-conductivity crust is smaller and, due to the exponential dependence of conductivity on temperature, the current in the crust will be localised in some separate overheated channels (by analogy to the thermal electric breakdown of dielectrics[21]). In this way one can imagine how the aforementioned surface warm spots are created. It is in these channels that the main energy will be released and hence they will act as natural centres of volcanism. Owing to such 'high quality' input of energy, the volcanoes should appear even at $W_{max} < W_{rad}$. The present mechanism of the volcanic activity excitation has advantages over the tidal mechanism where the main energy is released in the centre of a satellite and at high latitudes[22].

The slow non-synchronous rotation of the satellites (see below) and the maximum electrical field created between two diametrically opposite points mean that the volcanic channels have to be localised primarily in the low and middle latitudes. And indeed, all the known volcanoes and warm spots are at latitudes $\lesssim 30°$ (refs 2, 18). This new type of volcanism indicates that a new type of convection predominates in the crust: for example, the overflowing onto the surface lavas hardens; the crust sinks due to the additional load and its lowest layer melts and then it flows up-wards through volcanoes. The present rate of Io's resurfacing due to volcanic rocky deposits may be 1 mm or more per year[23]. So during 4,000 Myr all the satellite matter should make more than one convective cycle. This convection does not exclude the global convection, when because excess energy is liberated in the low latitudes the surface matter shifts to the poles (in the case of the tidal mechanism, in the opposite direction). Modern conceptions on the Earth's tectonism, suggest that the collision of the surface plates near the poles give rise to mountains. The towering mountains in the polar regions (see above) do exist.

The comparison of W_{max} with W_{rad} for galilean satellites suggests that all of them had initially large amounts of ice but the inner satellites received so much excess energy that the ice was lost.

Intensive explosions from Io's volcanoes accelerating stones up to, and more than 1 km s^{-1} imply the Plinian type of eruption and indicate that a considerable amount of volatiles exist inside Io, contrary to the ancient lunar volcanism which was volatile-poor and so developed mainly basaltic floods[24]. To eject bombs at a velocity 0.5 km s^{-1} the volcanic activity products must contain on the average ~10% gases by weight[25]. An estimated oxygen concentration in Io's plasma torus seems to be much more than the sulphur concentration[26], so the main gas component ejected is probably not SO_2, which aerodynamically is not the best driving medium, but water, as in the Earth volcanoes (so it would be worth attempting to find H_2O and OH in the torus and, essentially, in volcano plumes). Practically all ejected gases escape from Io. Taking into account the present rocky deposition rate 1 mm yr^{-1} (density $\rho = 3$ g cm^{-3}), one should obtain the ice layer ($\rho = 1$ g cm^{-3}) lost during 4,000 Myr as thick as ~1,000 km. Thus it seems reasonable to regard the ancient Io as a Ganymede-like icy body, and its modern rocky volcanic face as a recent acquisition.

Table 1 presents data for Amalthea and 'Amalthio' (an imaginary satellite having Io's parameters but located on Amalthea's orbit). Amalthea's orbit is interesting for three reasons: (1) it is very close to the Roche limit of Jupiter, (2) it practically coincides with the orbit where the value of $W_{max} (\gg W_{rad})$ becomes greatest, and (3) it lies well inside $4–5R_J \approx$ the radius of proto-Jupiter when it set into hydrostatic equilibrium after its rapid collapse caused by the molecular hydrogen dissociation[27] (here proto-Jupiter became sufficiently dense compared with the previous stage when it filled its critical Roche lobe, $R \approx 500R_J$ ref. 28). It would be natural to assume that five, not four, 'galilean' satellites existed initially, but Amalthio has been literally incinerated by Jupiter-Thunderer—by the unipolar electric currents (even now Amalthea is warmer than was expected[18]) and by the hypersonic and radiative ablation. It is difficult to elucidate the relative role of these factors in the

formation of the present Amalthea. It must be surely a remnant of a dense metallic core of Amalthio, strongly elongated by the ablation and bearing traces of the latter. But at some stage, matter could be dispersed by volcanic eruptions. As in the volcanism type under consideration the energy is released directly in the volcano throat, its transformation into the energy of eruptions must have a great efficiency, up to a few per cent. The energy of Amalthio's matter dispersion into space is $\sim GM_s^2/R_s = 3\times10^{29}$ J, so at the energy input $W_{max}\approx10^{14}$ W it takes a few thousand Myr to disintegrate the satellite. In reality, the convection and probably the rotation of young Jupiter were much more intensive[29], so that its past magnetic field was certainly far stronger than at present. $W_{max}\propto B^2$ and the duration of transformation of Amalthio in Amalthea could be below 1,000 Myr. Some of Amalthio's fragments probably created the inner ring around Jupiter.

The idea of the five large satellites and their initial abundance of ice contradicts somewhat the commonly accepted 'micronebular' hypothesis of satellite condensation in the gaseous disk which surrounded hot proto-Jupiter[29], but it accords with my cosmogonical conception which considers the birth of the Solar System as a limiting case of a close binary star origin[30]. The main planetary population of our system consists of Moon-like bodies[28]. They were accreting inside proto-Jupiter filling its critical Roche lobe, in the vicinity of the first lagrangian point, when proto-Jupiter's matter was overflowing onto the Sun, and they were lost one-by-one owing to a rapid decreasing of proto-Jupiter's mass. When the outflowing was completed by cooling the vast and rarefied proto-Jupiter, the last moons (having the great ice abundance) were not able to free themselves and so remained in Jupiter's power as the galilean satellites.

The electric current flows through the satellites across the magnetic field, supplying them with an excess angular orbital momentum[12]. The orbital momentum doubles in

$$\Delta t \approx M_s v_{orb} D/2I_{max}BR_sD = 1.3\times10^{-12}v_{orb}\rho_sR_s/B^2\ \text{yr} \quad (1)$$

This time is small for little Amalthea (Table 1) and its existence on this orbit, so close to the synchronous one ($D_{syn}=1.6\times10^5$ km), would be stranger had it not been a much larger Amalthea in the past. (Amalthea's behaviour may be strongly modified by its own magnetic field of a ferromagnetic nature.)

The nonhomogeneity of the Ampere force $I\times B$ causes the axial rotation of a satellite to gain angular momentum. Some nonhomogeneity is created by the discrete current concentration in volcano throats. The main effect is probably caused by dipole inhomogeneity of the magnetic field: the greater force acts on the hemisphere facing Jupiter. So the satellite's axial rotation ω will be slower than its orbital rotation n. The torque created by the dipole component of the Jovian field is estimated as

$$T_B \approx I\frac{dB}{dD}R_s^3 = -3IBR_s^3/D \quad (2)$$

This torque is balanced by the torque applied by Jupiter to the tidal bulges of the satellite[31]

$$T_Q = \frac{3}{2}\frac{kGM^2R_s^5}{D^6}\sin 2\delta \quad (3)$$

Where k is the Love number (for the Moon $k=0.02$, for Mercury $k=0.05$ (ref. 31), we assume $k=0.02$), δ is the lag angle of the bulges due to the internal friction in the satellite ($\delta\approx0.005$ for the Moon). In strongly asynchronous rotation, the Earth-type planets behave as solid bodies and $\delta\approx$ constant is a good approximation. But in fully synchronous rotation ($n=\omega$) there must be $\delta=0$, so that $\delta\to0$ at $n-\omega\to0$. Physically it is quite understandable: under the long-time load the creep begins to work and the matter flows, thus reaching the hydrostatic equilibrium. For fluids $\delta\propto(n-\omega)$ (ref. 32). We assume $\delta=0.005(n-\omega)/(n-\omega)_0$, here $(n-\omega)_0=2\pi/P_0$ (a similar dependence has been accepted elsewhere[33–35]). Equating the torques (2) and (3) we find at $I=I_{max}$, the values $n-\omega=2\pi/\Delta P$, where ΔP is the period of the 'lost' revolution. At $P_0=10h$ the rotation of the galilean satellites relative to the synchronous position makes up ~1—10 arc min yr^{-1} (the equatorial velocity ~10–100 m yr^{-1}) (see Table 1). This is a rather small but detectable value, the measurement of which will allow us to specify the models of the satellites and the details of their interaction with Jupiter and its environment.

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Evidence of SO_2 on Io from UV observations

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Gaseous SO_2 was discovered on Io, the innermost galilean satellite of Jupiter, by the IRIS IR Michelson interferometer[1] on board Voyager 1 in March 1979. This SO_2 is likely to be associated with eruptions of the seven active volcanoes discovered at the same time by the Voyager cameras[2]. The UV spectrum of the solar light reflected by the surface of Io should be modified by the transmission $\bar{S}(\lambda)$ of a SO_2 atmosphere and by the reflectivity $r(\lambda)$ of the surface. Both gaseous SO_2 and solid[3] SO_2 present a deep absorption band around 290 nm. We have examined a published spectrum of Io taken in 1978 by the IUE Earth-orbiting telescope[4], and found an absorption dip centred around 290 nm in the albedo curve. We attribute this dip to gaseous SO_2, with a possible contribution of solid SO_2. However, the absorption dip is smaller than if the gaseous SO_2 quantity measured by Voyager 1 in the IR were uniform. It can be better represented by an SO_2 frost controlled gaseous atmosphere and a temperature distribution of Io's surface compatible with observations.

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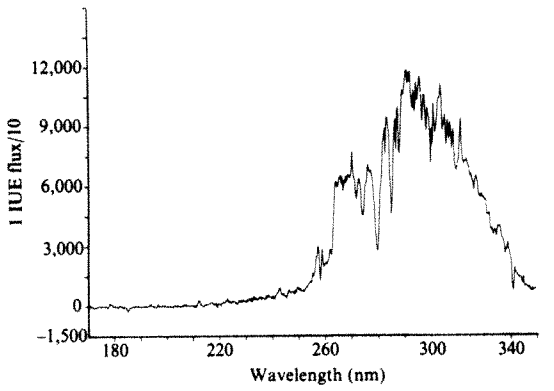


Fig. 1 The UV spectrum of Io as measured in 1978 with the orbital telescope IUE[4].

The surface of Io, even when covered with SO_2 frost, probably does not have the same UV optical characteristics as pure SO_2 frost obtained in the laboratory, as other materials are certainly present at the surface. To conduct a quantitative analysis, we have considered successively two extreme cases: either the surface reflectivity of Io does not show the absorption dip of pure SO_2 frost (which does not exclude its presence) or it reproduces it exactly (and therefore indicates its presence).

The average surface temperature of Io on the dayside is $\simeq$127 K, but some 'hot spots' exist where the temperature is probably as high as 290 K (ref. 5). This high temperature, which yields a high surface IR radiance, allowed the identification of an SO_2 absorption feature at 1,350 cm^{-1}. The vertical column density N is estimated to be 0.2 cm atm (ref. 1) which corresponds to a surface pressure of 1.0×10^{-4} mbar. This value is close to the vapour pressure of SO_2 frost at $T=129$ K (1.4×10^{-4} mbar). The similarity of these numbers led to suggestions that gaseous SO_2 content in Io's atmosphere is controlled by SO_2 frost. This idea is also supported by the recent identification of absorption due to SO_2 frosts in ground-based IR reflection spectra[6,7].

The geometric albedo of Io has been measured down to 320 nm from ground-based observations[8] and is displayed in Fig. 2. Further in the UV, the spectrum of Io in Fig. 1 has been measured with IUE[4], as was the spectrum of Mars. We derive the geometric albedo G(Io) below 320 nm from these data by using the relation:

$$\frac{G(\text{Io})}{G(\text{Mars})}=C\frac{\text{Spectrum (Io)}}{\text{Spectrum (Mars)}}$$

where C is a constant of proportionality which depends on the IUE observing conditions. The geometric albedo of Mars is obtained from measurements by OAO 2 (ref. 9), which is rather flat in this region (from 0.051 at 240 nm to 0.040 at 330 nm). The value of C was found to be 0.5 by matching the IUE albedo and the ground-based albedo at 330 nm. G(Io) is displayed in Fig. 2 and shows a pronounced dip, centred near 290 nm, reminiscent of the absorption coefficient K_λ of gaseous SO_2 also shown in Fig. 2. The vertical optical column density of 0.2 cm atm represents a vertical optical thickness $\tau_v(\lambda)=k_\lambda N$ of 6×10^{-3} at 340 nm and 3.5 at 280 nm. As only the integrated albedo on the surface is available, we computed the averaged transmission $\bar{S}(\lambda)$ of various SO_2 atmosphere models, assuming that the temperature of SO_2 frost controlled the gaseous SO_2. The vapour pressure ρ of gaseous SO_2 over frost is a function of surface temperature T (ref. 10)

$$\log_{10}\rho(\mu\text{ bar})=-1{,}843.04/T+13.7164$$

and the vertical column density is $N(\text{cm atm})=1.96\,\rho(\mu\text{ bar})$. Given a temperature distribution $T(\theta)$ as a function of solar zenith angle θ, the transmission $S_\lambda(\theta)$ of the atmosphere to reflected sunlight at 0° phase angle is $S_\lambda(\theta)=\exp[-2k_\lambda N(\theta)/\cos\theta]$. If $r(\lambda)$ is the normal reflectivity of Io's surface in the absence of atmospheric absorption, the geometric albedo at 0° phase angle is:

$$G(\lambda)=r(\lambda)\int_0^{\pi/2}\cos^{n+1}\theta\,S_\lambda(\theta)\,d(\cos\theta)=r(\lambda)\bar{S}(\lambda)$$

which defines the equivalent transmission $\bar{S}(\lambda)$. n is an index representing the limb darkening effect. However, we present here results only for $n=0$, as the dependence on θ is strongly dominated by the function $T(\theta)$. We have computed the equivalent transmission $\bar{S}(\lambda)$ for various temperature distributions $T(\theta)$ and have compared it with the measurement $G(\lambda)$ of Fig. 2. We take first a model for $r(\lambda)$ below 340 nm, with no SO_2 frost absorption dip, as a linear extrapolation of the trend observed above 340 nm. At 240 nm, where SO_2 absorption coefficient is small, this model (dashed line on Fig. 2) coincides with the measured $G(\lambda)$.

First we considered uniform temperature distribution. With a temperature $T=127.2$ K giving $N=0.2$ cm atm, we find $\bar{S}(340\text{ nm})=1$ (negligible absorption) and $\bar{S}(280\text{ nm})=2\times10^{-4}$ (total absorption). As $r(\lambda)$ must be <1, it implies that the albedo should be effectively zero at 280 nm. In fact, the non-zero albedo measured at 280 nm implies either a very low uniform value of $N(\simeq0.014$ cm atm corresponding to $T=117.4$ K) or a non-uniform distribution of the temperature and SO_2 column density.

We then considered models of the form

$$T(\theta)=T_s-\Delta T(1-\cos^m\theta)$$

where T_s is the temperature at the sub-solar point, $T_s-\Delta T$ the temperature at the terminator, and m a parameter describing how fast $T(\theta)$ decreases from T_s to $T_s-\Delta T$. For each value of T_s and m, a value of ΔT can be found which satisfies the observational constraint $\bar{S}(\lambda)\simeq0.50$ at 290 nm. The value of $m=1/4$, which provides a good empirical representation on

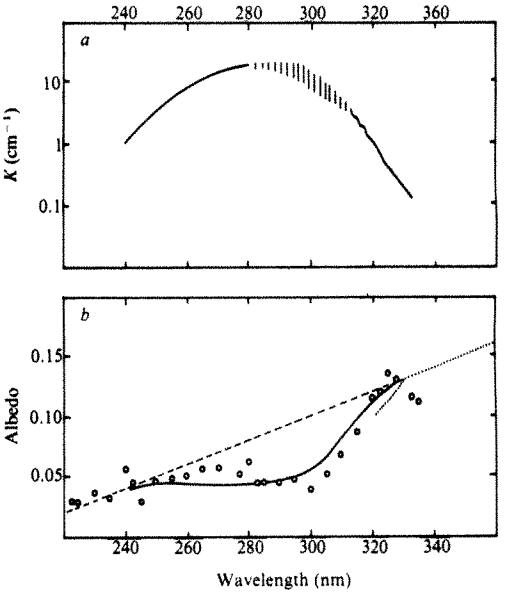


Fig. 2 *a*, The absorption coefficient K, of gaseous SO_2 as a function of wavelength[10]. There is a pronounced band structure between 280 and 320 nm which is represented schematically only. *b*, Geometric albedo of Io as a function of wavelength. Dotted line, ground-based measurements[8]. The linear extrapolation of ground-based measurements below 330 nm (dashed line) represents surface reflectivity variations $r(\lambda)$, if the SO_2 frost signature is ignored. ○, The measurements derived from spectrum of Fig. 1, spectrum of Mars and the known albedo of Mars. The solid line is the result of a computation of the transmission of a saturated SO_2 atmosphere (averaged over the disk of Io) above SO_2 frost, containing 0.2 cm atm at the sub-solar point, and a temperature variation compatible with IR measurements.

Mercury's temperature profile, would imply small values of T_s and large values of ΔT: $T_s = 123$ K and $\Delta T = 60$ K, which do not fit the IRIS data of $T_s = 127$–130 K, and $T = 110$ K at the evening terminator (ref. 1 and J. Pearl, personal communication). For $m = 1$, a good fit of $G(\lambda)$ is obtained with $T_s = 127$ K (corresponding to $N = 0.19$ cm atm) and $\Delta T = 34$ K and is represented in Fig. 2. This is quite reasonable, as we expect the morning terminator to be colder than the evening terminator. We, therefore, conclude that there was, at the time of IUE observations, a quantity of gaseous SO_2 comparable to the quantity detected by Voyager 1, if the temperature distribution was represented by $T = T_s - \Delta T(1 - \cos\theta)$, and if the surface reflectivity $r(\lambda)$ behaves as assumed, without any UV SO_2 frost signature.

Now we consider the other extreme case where the surface of Io has a reflectivity $r(\lambda)$ reproducing exactly the UV SO_2 frost signature. Indeed, it has been suggested[3] that the decrease of geometric albedo from 330 to 320 nm (ground observations) may be due to SO_2 frost. A laboratory reflectivity spectrum of pure SO_2 frost at 130 K was recently obtained[12], showing a decrease of a factor of 28 between 325 and 275 nm. Such a decrease is clearly absent from IUE observations, which would mean in this case that only a fraction $A_f \simeq 0.24$ of the planet is covered with SO_2 frost, the rest being covered with a background material with a reflectivity $\alpha_B \simeq 0.064$. This is obtained by fitting IUE observations at 275 and 325 nm with a combination of background material, pure SO_2 frost and gaseous SO_2 over this SO_2 frost. In such a case, the absorption due to gaseous SO_2 at 275 nm would be ~12%, averaged on the whole disk.

The UV spectrum of Io shows evidence of the UV SO_2 band centred at 280 nm. To what extent this band has a contribution of SO_2 frost or is only due to gaseous SO_2 is still an open question. It could be solved by resolving the spectral structure of the gaseous SO_2 between 280 and 310 nm, absent from the pure SO_2 frost spectrum. However, our model of the temperature-controlled SO_2 atmosphere has shown that the variations of $\bar{S}(\lambda)$ are much smaller than the variations of the gaseous SO_2 absorption coefficient k_λ, requiring, therefore, accurate measurements. The monitoring of the geometric albedo spectrum, from ground observations, but more conveniently from IUE, would provide a record of changes in the atmospheric SO_2 content. This SO_2 content is a result of volcanic activity, extent of SO_2 frost deposit, temperature profile, and escape to the ionised torus discovered by Voyager 1 at the orbit of Io[13]. It would be of particular interest to observe the rise and decay times associated with a large surge of activity.

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Pioneer magnetometer observations of the Venus bow shock

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Venus has a bow shock in many respects similar to that of the Earth. The Venus bow shock was probed twice by Mariner 5, once by Mariner 10, Venera 4, and Venera 6 (refs 1–4 respectively) and repeatedly by Venera 9 and 10 (ref. 5). The Pioneer Venus orbiter is now circling Venus in a 24-h elliptical orbit which crosses the bow shock twice a day[6]. Here we report the somewhat surprising result that the Venus bow shock is not as strong as the terrestrial bow shock.

The orbit of the Pioneer Venus spacecraft is inclined at 105° to the ecliptic plane with periapsis at a latitude of 17°N and at an altitude of ~150 km. This orbital geometry provides shock crossings principally at solar zenith angles of greater than 60°. Figure 1 shows several recent shock crossings as recorded in the change in magnetic field strength. The shocks are ordered from top to bottom in terms of increasing θ_{BN}, the angle between the interplanetary field direction and the model[7] shock normal. The angle, θ_{BN}, has been found to order the observed structure of the terrestrial bow shock and the waves upstream of the shock[8]. Similar control is observed here but it is not yet clear whether the control is identical to the terrestrial case.

On each panel for $\theta_{BN} \geq 50°$ we have listed the field jump in the plane of the shock as determined from 1-min averages again using a shock model to determine the direction of the local shock normal. The average jump is 2.72 ± 0.16. We have repeated this analysis for nine terrestrial shock crossings at similar solar zenith angles and angles, θ_{BN}, observed by the ISEE spacecraft during December 1977. The average field increase in the plane of the terrestrial shock was 3.37 ± 0.07, almost 25% stronger. Further, the overshoot in field strength so obvious in the terrestrial shock[9] can barely be recognised in the typical Venus shock crossing.

Figure 2 shows that the weakness of the Venus shock extends over all solar zenith angles sampled to date. Here we plot the jump in the total field, not just in the plane of the shock, for 83 bow shock crossings in the range of solar zenith angles from 60° to 110°. The upper dashed line is the jump in field strength predicted by a gasdynamic simulation of the solar wind–Venus interaction.[10] The Mach number of the incoming flow is 6 and the ratio of specific heats is 5/3 for this simulation. The jump in total field for our nine comparison terrestrial shocks was 3.11 ± 0.10 at a median solar zenith angle of 72°. We note that the observed field jump at the Venus bow shock does decrease with increasing solar zenith angle as in the gasdynamic simulation.

The reason for the weakness of the Venus bow shock relative to the terrestrial bow shock is not obvious. In the gas-dynamic simulation one can decrease the amplitude of the shock jump by increasing the ratio of specific heats from 5/3 to 2, but it is not evident why this ratio would be different for the two planets. We would not expect the Mach number to be significantly different at the two planets.

If the difference in shock strengths were due to some radial variation of solar wind properties we would expect that the shock at Mercury would be even weaker than the Venus shock. this is clearly not so of the two perpendicular shocks seen by Mariner 10 (refs 4, 12) one was comparable to the terrestrial shock in strength and one to the Venus shock.

We have restricted our comparison to a similar range of solar zenith angles, and in this range of angles the shock shape is the same for the two planets[7]. Thus we cannot attribute the difference in shock strengths to differing shock geometries. We must conclude that there is something basically different about the solar wind interactions with the two planets. Perhaps this difference is the existence of photo-ion pickup[13] or charge exchange with the Venus geo-corona[14]. A similar conclusion is suggested by the unexpected closeness of the bow shock to Venus[7,15].

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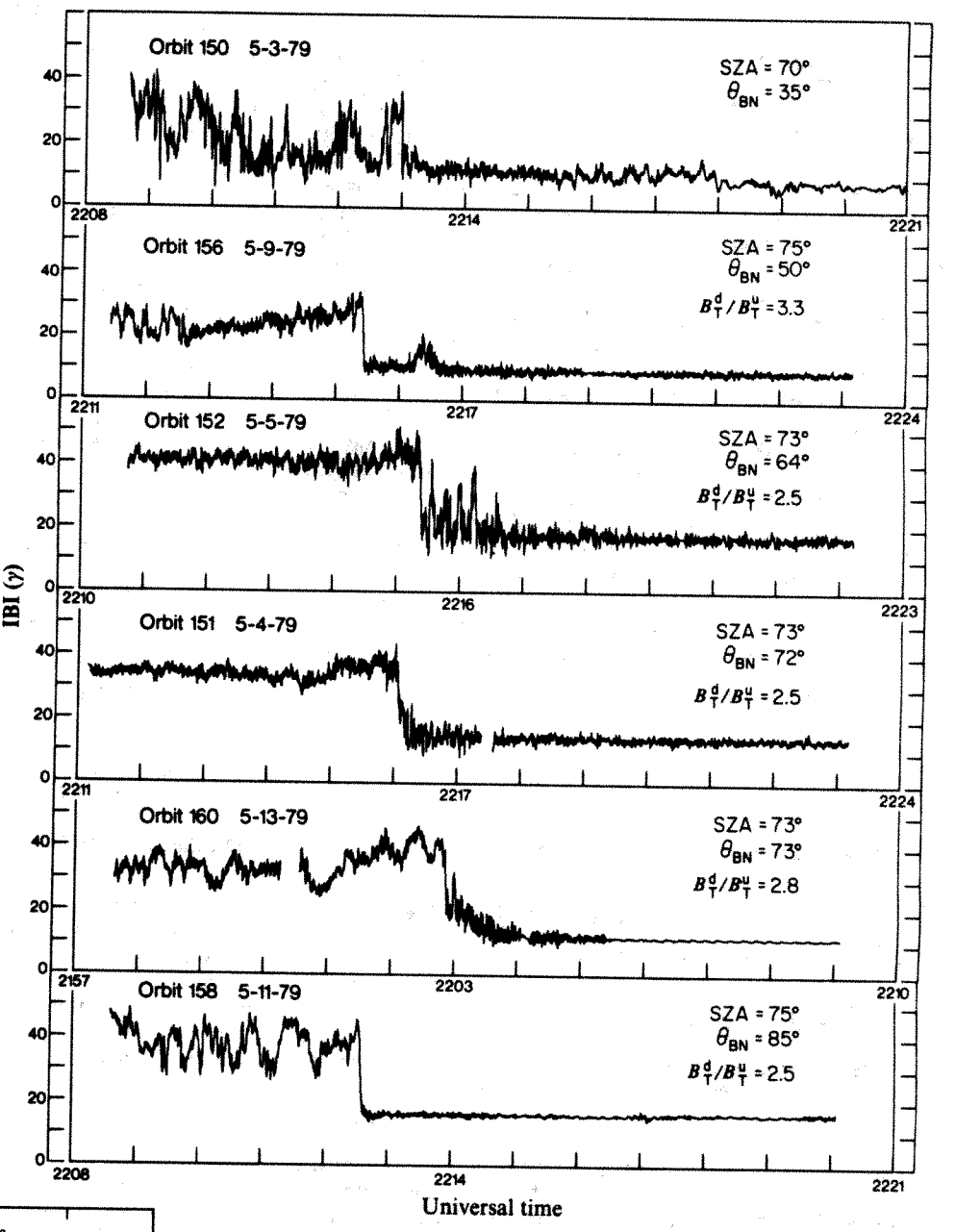


Fig. 1 Magnetic field strength versus time for six Pioneer Venus bow shock encounters. Sample rate is 4 samples per second. SZA is solar zenith angle, that is the angle between the Venus–Sun and Venus–spacecraft line. The angle θ_{BN} is the angle between the upstream magnetic field and a model shock normal. B_T^u is the 1-min average upstream, (interplanetary) magnetic field projected into the plane of the shock; B_T^d is the downstream magnetic field projected into the shock plane.

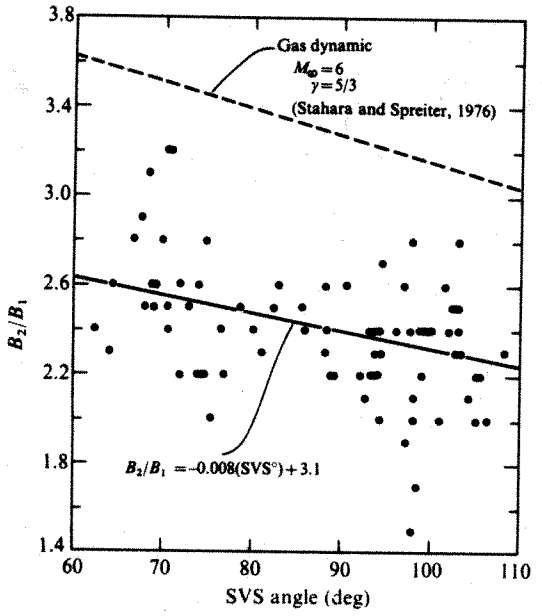


Fig. 2 The ratio of the downstream total field strength to the upstream field strength as function of the Sun–Venus–satellite or solar zenith angle. The solid line is the best least-square fit to the data, the dashed line is taken from a gas-dynamic numerical simulation.

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Heterogeneous photocatalytic reduction of dichromate on n-type semiconductor catalysts

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Photocatalytic and photoelectrosynthetic processes[1] are interesting from the point of view of solar energy utilisation. These processes can be developed using semiconductor electrochemistry[1-9]. The fixing of carbon dioxide on semiconductor powders[10] and amino acids synthesis on platinised TiO_2 (ref. 11) are good examples. Here we report the photocatalytic reduction of dichromate to Cr(III) on n-type semiconducting powders. This is important as dichromate is a harmful substance, and has to be treated with a reducing agent such as sodium thiosulphate to give Cr(III) in the first step of the waste treatment.

The basis of the photocatalytic reduction of dichromate is that the reaction is composed of the following two electrochemical processes;

$$2Cr_2O_7^{2-} + 28H^+ + 12e^- \rightarrow 4Cr^{3+} + 14H_2O \quad (1)$$

$$6H_2O \rightarrow 3O_2 + 12H^+ + 12e^- \quad (2)$$

Overall:

$$2Cr_2O_7^{2-} + 16H^+ \rightarrow 4Cr^{3+} + 8H_2O + 3O_2 \quad (3)$$

$$\Delta G^0_{298} = -115.8\ \text{kJ}$$

Although the overall reaction is thermodynamically feasible, dichromate is stable in aqueous acidic solutions. As the electrochemical oxidation of water usually requires a high overpotential, the difficulty of water oxidation seems to be responsible for the stability of dichromate ions in solution. As n-type semiconductors such as TiO_2, WO_3, α-Fe_2O_3 and $SrTiO_3$ can photosensitise the oxidation of water[12,13], these must be efficient photocatalysts for the reduction of dichromate ions.

It was found that the rate of the photocatalytic reduction follows equation (4);

$$-dC/dt = k\sqrt{C} \quad (4)$$

Table 1 shows the results obtained for different types of photocatalysts. The concentration of dichromate was determined by colorimetric analysis using diphenylcarbazide as a colour former. Table 1 shows that WO_3 was the most active. The quantum yield for the photocatalytic reduction on WO_3 at $\lambda = 400$ nm was 0.02, as determined by ferrioxalate actinometry.

Figure 1 shows an absorption spectrum of a dichromate solution and photocurrent spectra of WO_3 and TiO_2 electrodes in 0.5 M H_2SO_4. The absorption spectrum of the dichromate solution matches fairly well those of the semiconductor photocatalysts. Therefore, the light efficiency for photoexcitation of carriers is seriously weakened at the catalyst surface depending on the concentration of the solution and the thickness of the solution layer around the catalyst. The molar absorption coefficient of the dichromate solution at $\lambda = 350$ nm, where the maximum absorption was observed, was determined to be 2.24×10^3 M cm^{-1}, and that at $\lambda = 400$ nm, where WO_3 shows the maximum photoresponse, to be 4.4×10^2 M cm^{-1}.

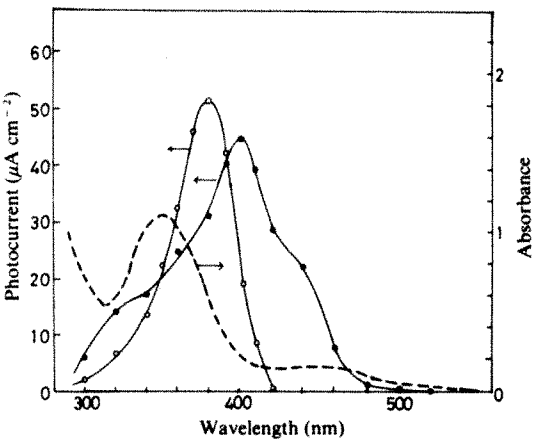


Fig. 1 Absorption spectrum of 5×10^{-4} M $K_2Cr_2O_7$ in 0.5 M H_2SO_4 and photocurrent spectra of WO_3 and TiO_2 electrodes at 0.8 V versus SCE in 0.5 M H_2SO_4. ●, WO_3; ○, TiO_2. These spectra are uncorrected for intensity variation of the lamp-monochromator output, with the drop off at shorter wavelengths attributable to the lamp. The WO_3 electrode was prepared by thermal decomposition of WCl_6 (ref. 14) and the TiO_2 electrode by thermal oxidation of a titanium plate[15].

The utilisation of solar energy in the photocatalytic reduction on WO_3 was investigated for dilute static dichromate solutions and for a concentrated agitated solution in the same way as described in Table 1. The results are given in Table 2. In spite of the high absorptivity of dichromate, the photocatalytic reduction was possible even for the solution conventionally used as a cleaning solution (no. 3 of Table 2) if that solution was stirred.

Figure 2 shows a steady-state current–potential curve of an illuminated WO_3 electrode in 0.5 M H_2SO_4 with and without dichromate ions. The polarisation curves were obtained with the illumination intensity adjusted to give the same anodic photocurrent at 1.2 V in both electrolytes with and without dichromate. In the solution without dichromate, the photosensitised oxidation of water occurred at potentials >0.3 V versus SCE, in agreement with the results already reported[16]. As Fig. 2 shows, a cathodic current peak appeared at about 0.55 V when the electrode was illuminated in the dichromate solution. If there was no illumination, the cathodic current due to the

Table 1 Photocatalytic reduction of dichromate on different types of semiconductors*

Catalyst†	λ_{th}‡ (nm)	Purity (%)	Surface area ($m^2\ g^{-1}$)	Amount of catalyst (g)	Decrease for 1 h (%)§	Rate constant, k ($mol^{1/2}\ dm^{-1/2}\ h^{-1/2}$)
TiO_2, rutile	410	99.9	9.5	0.2, 0.5, 0.8	19	1.4×10^{-2}
reduced rutile‖	410		8.6	0.2	37	2.9×10^{-2}
TiO_2, anatase	410	99.9	8.0	0.2, 0.5	13	9.5×10^{-3}
WO_3	520	99.9	5.0	0.2, 0.4, 0.6	48	3.9×10^{-2}
α-Fe_2O_3	620	99.99	3.4	0.3, 0.5, 1.1	9	3.5×10^{-3}
$SrTiO_3$	390	99.9	0.67	0.2, 0.6	4	1.3×10^{-3}

* Dichromate solution, 5×10^{-3} M $K_2Cr_2O_7$ in 0.5 M H_2SO_4. Glass reaction vessel, 15 mm diameter, 45 mm height. Solution volume, 5 ml. Light source, 1 kW xenon lamp. The amount given in the table was added to the solution, and light of ~10 mm diameter focused onto the agitated suspension under open air. The solution was agitated with a magnetic stirrer so that no appreciable amount of the catalyst remained on the bottom of the reaction vessel.

† Manufacturers: rutile, Fuji Titanium; anatase, Merk; WO_3, α-Fe_2O_3, and $SrTiO_3$, Mitsuwa Pure Chemicals.

‡ The threshold wavelength beyond which no photoresponse is observed[10].

§ Almost the same value was obtained for the corresponding different amount of catalyst given.

‖ Reduction in H_2 at 600 °C for 3 h.

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Table 2 Photocatalytic reduction of dichromate on WO_3 with sunlight

No.	Concentration of solution (mM) and volume used (ml)	Diameter of* vessel (mm)	Reaction mode	Amount of catalyst (g)	Solution above† the catalyst (mm)	Reaction time (h)	% of reaction
1	0.5‡×5	40	statis	6	2–3	0.5	<99
2	5‡×5	80	static	10	<1	1	68
3	62.5‡×10	40	agitated	2		9	88

* Experiments were carried out in glass vessels of 40 mm diameter, 30 mm high, or of 80 mm diameter, 10 mm high covered with saran wrap.
† In the static mode of the reaction, the catalyst stayed on the bottom of the vessel.
‡ The solutions in nos 1 and 2 were prepared by dissolving dichromate in 0.5 M H_2SO_4 and that in no. 3 into 18 M H_2SO_4.

reduction of dichromate, which occurs at potentials <0.8 V with superposition of the oxygen reduction, was much smaller than that obtained under illumination and showed no current peak. The cathodic process in the dichromate solution was also enhanced at TiO_2 electrodes, although to a lesser extent than with the WO_3 electrode. No enhancement was observed at a single crystal $SrTiO_3$ electrode.

Figure 2 suggests that the expected electrochemical mechanism prevails in the photocatalytic reduction of dichromate, because the cathodic process of the dichromate reduction occurs at potentials greater than the onset potential of the oxidation of water. According to equations (1)–(3), the reduction to Cr(III) should be accompanied by the evolution of oxygen, but no appreciable evolution was observed in the experiments shown in Tables 1 and 2. However, small bubbles evolved intermittently from the surface of a WO_3 layer packed half way up a reaction vessel (15 mm diameter, 45 mm height) when the catalyst was kept still and xenon light was focused onto a zone of the packed $WO_3/10^{-3}$ M dichromate solution interface.

Where the photocatalytic reaction is governed by an electrochemical mechanism, a high reaction rate will be expected if the catalyst gives high cathodic current at potentials where the photo-anodic reaction occurs. In the case of WO_3, the cathodic process is largely enhanced by illumination as stated above, so that the highest reaction rate must be achieved. The rate of the cathodic reaction seems to be governed by both the degree of the enhancement by illumination and electrocatalytic activity of the material itself in the dark. The order of the catalytic activity shown in Table 1, that is, WO_3 > rutile > anatase > α-Fe_2O_3 > $SrTiO_3$, seems to reflect such a situation.

A cathodic process is not usually enhanced at n-type semiconductors, as electrons as the major carrier participate in this process. Therefore, the results obtained here are not popular in semiconductor electrochemistry, although a similar phenomenon was observed on photocatalytic oxidation of methanol on TiO_2 (ref. 17). To clarify the mechanism of the photocatalytic reduction of dichromate, this problem has to be clarified. These preliminary results indicate that photoadsorption of dichromate may have some role in the enhancement of the cathodic process.

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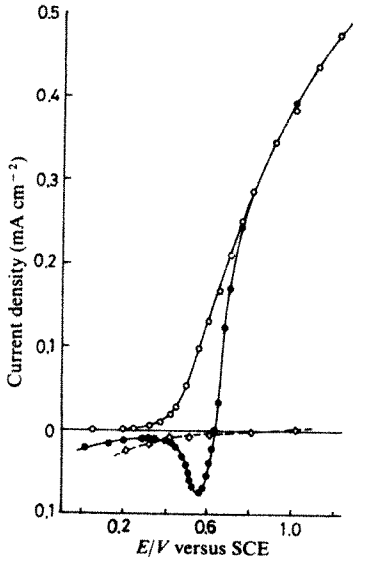


Fig. 2 Current–potential curves of a WO_3 electrode in 0.5 M H_2SO_4. ○, Under illumination in oxygen-free 0.5 M H_2SO_4. ●, Under illumination in oxygen-free 0.5 H H_2SO_4 containing 5×10^{-3} M $K_2Cr_2O_7$. ◇, In the dark in aerated 0.5 M H_2SO_4 containing 5×10^{-3} M $K_2Cr_2O_7$.

Tropospheric ozone and climate

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Analysis[1] of the observed tropospheric ozone distribution has revealed that concentrations in the Northern Hemisphere were larger than in the Southern Hemisphere. This finding and the earlier identification of the necessary photochemical reaction scheme[2] has led Fishman and Crutzen[1] to suggest that an appreciably larger *in situ* photochemical source of tropospheric ozone may exist in the Northern Hemisphere than in the Southern Hemisphere and to speculate that anthropogenic activity might be responsible for a considerable fraction of the larger ozone concentrations in the Northern Hemisphere. In the present study, we examine the influence of tropospheric ozone on climate. Ozone is an optically active gas, which absorbs and emits terrestrial IR radiation in the 8–10-μm region and absorbs solar radiation in the UV and visible. Hence, a change in ozone concentrations will perturb the radiative energy budget of the Earth–atmosphere system which may in turn perturb the climate.

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Table 1 O_3 and CO source strengths (in molecules $cm^{-2} s^{-1}$)

	Northern Hemisphere	Southern Hemisphere	Ref.
Carbon monoxide			
Industrial CO emissions	19×10^{10}	4×10^{10}	7
CO from methane oxidation	$4–8 \times 10^{10}$	$4–8 \times 10^{10}$	5
Ozone			
From *in situ* production	$12–32 \times 10^{10}$	$5–14 \times 10^{10}$	5
From stratospheric injection	$5–8 \times 10^{10}$	$3–4 \times 10^{10}$	8–10

The ozone–climate problem has been studied mainly by radiative–convective models (see review in ref. 3). The recent study by Ramanathan and Dickinson[4] revealed that a uniform percentage decrease of ozone in the troposphere has about the same net radiative cooling effect on the surface–troposphere system as the same percentage decrease of ozone in the stratosphere. This result is surprising as only about 10% of all ozone resides in the troposphere. The relatively strong sensitivity of the terrestrial radiative energy balance to a tropospheric ozone change is caused by several factors[4], the principal one being that the integrated 9.6-μm band opacity of ozone is a function of atmospheric pressure. More specifically the line halfwidths of the individual rotational lines of the 9.6-μm band are linearly proportional to pressure, at least below 30 km. Consequently, although only ~10% of ozone is present in the troposphere, the IR opacity of tropospheric ozone is nearly the same as that of stratospheric ozone.

Our study will focus on the following aspects of the tropospheric ozone–climate problem: (1) interhemispheric differences in radiative heating of the surface–troposphere system caused by observed interhemispheric ozone distribution differences as reported by Fishman and Crutzen[1]; and (2) the sensitivity of surface–troposphere radiative heating to tropospheric ozone changes. The effects are estimated as a function of latitude and season.

Several recent studies have hypothesised that ozone concentrations may have increased substantially by industrial activities in the Northern Hemisphere and will continue to grow as human activity increases[1,5,6]. Admittedly, except in highly polluted air, it is difficult to prove, by direct observations the *in situ* photochemical production of tropospheric ozone from the oxidation of carbon monoxide and hydrocarbons in the presence of nitric oxide, because of the slow production of ozone in background air[5]. Table 1 summarises some of the studies which suggest that the amount of *in situ* photochemical production of ozone may be considerably larger than injection from the stratosphere[5,7–10] and Table 2 presents data that show the asymmetry in both the CO and O_3 distributions between the hemispheres[5,7,11–13].

Note that there are several reports which either support or refute the importance of tropospheric ozone production[1,5,6,10,14–17]. An indication that tropospheric ozone production does indeed take place in the Northern Hemisphere remote troposphere has recently been obtained from an analysis of simultaneous observation of the concentrations of CO and O_3, generally showing a positive correlation between about 2 and 8 km in the atmosphere[11].

Of similar importance for the production of ozone in the troposphere is the presence of hydrocarbons or carbon monoxide together with nitric oxide. The anthropogenic inputs for both CO and NO are now estimated to be comparable to the natural sources[15–21]. As these emissions probably take place simultaneously, they are of special importance for tropospheric ozone production.

The indications support the view that the asymmetric distribution of ozone between the hemispheres may to a substantial degree be the result of human activities. However, there is no undisputable proof that tropospheric ozone concentrations have generally increased in the Northern Hemisphere over the past decades, as a sufficient long-term record of tropospheric ozone observations is not available.

We use the radiative transfer model described elsewhere[4] which extends vertically from the surface to 54 km. Eight latitude bands are considered from the Equator to 70°, equally spaced at 10° intervals. The model computes the vertical distribution of IR and solar fluxes, using observed Northern Hemisphere distributions of temperature, humidity, clouds at three levels, CO_2, CH_4, N_2O and O_3, and surface albedo as a function of latitude and month. The radiative fluxes computed from these observed distributions are referred to as unperturbed fluxes.

The perturbation calculations discussed here are brought about by prescribing varying amounts of ozone in the troposphere; the change in stratospheric temperatures from the observed value is computed from radiative equilibrium considerations. It was found, however, that the stratospheric temperature changes had a negligible influence on the present results. The distribution of IR and solar fluxes is computed next and the differences between the fluxes in the perturbed and unperturbed troposphere are computed. The flux difference at the tropopause denotes the change in the net radiative energy input to the surface–troposphere system. It is this quantity which is considered here.

The standard profile is based on the observed distribution of ozone concentrations up to 12 km in the Northern Hemisphere as a function of month and latitude. We used three perturbations to our 'standard' profile of tropospheric ozone: (1) the ozone concentrations below 12 km were prescribed according to their distribution in the Southern Hemisphere[1]; (2) ozone concentrations were arbitrarily halved at all levels from the ground to 12 km from the standard distributions; and (3) the standard ozone concentrations were arbitrarily doubled up to 12 km. The first case would simulate the present day heating difference between the two hemispheres solely because more ozone is observed in the Northern Hemisphere than in the Southern Hemisphere troposphere. The second case examines the consequences of the assumption that tropospheric ozone in the Northern Hemisphere has already doubled due to anthropogenic activities. The doubling of tropospheric ozone considered in the third case is a projection into future anthropogenic influence on this gas.

The latitudinal distributions of the annual average values of the surface–tropospheric heating for the three cases are shown in Fig. 1. Considering first the interhemispheric differences, the latitudinal distribution of the difference in radiative heating rates between the Northern and Southern Hemispheres is due mainly to the latitudinal distribution of corresponding ozone concentration differences. At high latitudes, there are also significant seasonal variations in the interhemispheric ozone differences which cause corresponding seasonal variations in interhemispheric heating rate differences (not shown in Fig. 1). For example, at 50°N we calculate interhemispheric heating rates of 0.61, 0.77, 0.48 and 0.35 $W m^{-2}$ for winter, spring, summer and autumn, respectively. This seasonal variability is considerably less at low latitudes. However, more tropospheric ozone measurements in the Southern Hemisphere are needed before attaching too much importance to these seasonal variations. From Fig. 1, the hemispheric and annual mean value of the

Table 2 Observed hemispheric asymmetry of integrated CO and O_3 in the troposphere

	Asymmetry (Northern/Southern Hemisphere)	Latitudinal domain	Ref.
Carbon monoxide	1.8	90° S–90° N	7
	1.4	53° S–67° N	11
Ozone	1.3	60° S–60° N	5
	1.4	53° S–53° N	11
	1.2	90° S–90° N	12
	1.3	30° S–30° N	13

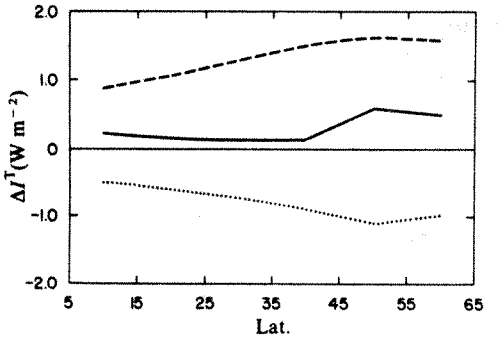


Fig. 1 Annual mean Earth–troposphere radiative heating (ΔI^T) resulting from tropospheric O_3 perturbations. Solid line, heating caused by interhemispheric O_3 differences; dotted line, heating caused by halving tropospheric O_3; dashed line, heating caused by doubling tropospheric O_3.

difference in the radiative heating rates between the Northern and Southern Hemispheres is about 0.3 W m^{-2}. This difference in radiative heating can be related to a corresponding difference in surface temperature, ΔT_s, by letting

$$\Delta T_s = \frac{\Delta I^T}{dI^T/dT_s} \quad (1)$$

where the numerator is the hemispheric average of the change in the energy input to the surface–troposphere system and the denominator is the climate sensitivity parameter which has been extensively discussed during the past decade or so. Values for this parameter[3,22] range between 0.75 and 2.5 W m^{-2} K^{-1}. We will choose the estimate derived by Ramanathan[23], dI^T/dT_s = 1.5 W m^{-2} K^{-1}, which is based on Wetherald and Manabe's[24] general circulation model climate sensitivity analysis. Introducing the difference of 0.3 W m^{-2} in heating between the hemispheres in equation (1), we obtain $\Delta T_s \approx 0.2$ K, that is, the Northern Hemisphere could be warmer than the Southern Hemisphere by about 0.2 K because of the larger amount of ozone in the Northern Hemisphere troposphere. Note that the Northern Hemisphere is indeed warmer than the Southern Hemisphere by abut 1.0 K (ref. 25). There are, of course, other inter-hemispheric differences, such as land and ocean distribution, cloud cover, snow and ice covered areas, and water vapour concentrations which together should be more important in explaining the observed temperature difference between the hemispheres than the differences in tropospheric ozone. Nevertheless, our calculations indicate that differences in ozone may contribute appreciably to the different observed mean temperatures. Applying equation (1) to the second and third cases shown in Fig. 1, we estimate that a halving of the tropospheric ozone concentrations may cool the surface by 0.5 K and that a doubling may warm it by 0.9 K.

We feel that considerations of man's alteration of the global climate must incorporate the possibility that tropospheric ozone concentrations may increase during the coming decades. Our calculations indicate that a doubling of the tropospheric ozone content, calculated by Logan *et al.*[6] to occur by the end of the next century, may increase surface temperatures by nearly 1 K. In a similar period of time a doubling of carbon dioxide in the atmosphere[26] will probably occur, which may result in a 2–3 K temperature increase[3]. A difference between these effects is that, as a result of shorter photochemical lifetimes, the heating resulting from increasing tropospheric ozone would be more concentrated in the Northern Hemisphere, whereas the heating by carbon dioxide would be distributed more evenly between the two hemispheres. It seems possible therefore, that the photochemical production of ozone in the troposphere may enhance the potential climatic consequences of future CO_2 increases by a substantial factor, in particular in the Northern Hemisphere.

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Project Kiwi One: an acoustic cross-section of the South Pacific Ocean

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The deep ocean sound channel is used to obtain very long range (typically >2,000 km) acoustic transmission[1] via totally refracted propagation paths (SOFAR propagation[2]). Such experiments can therefore determine the acoustic transmission properties of large areas of ocean[3]. Those acoustic propagation experiments can be used to locate major oceanographic changes[4] and to identify specific water masses[5]. A 10,000-km underwater sound transmission experiment conducted between New Zealand and Peru to obtain an acoustic cross-section of the South Pacific Ocean is described here. Three distinct regions of transmission loss were found. The highest attenuation, which is attributed to Antarctic intermediate water, occurred in the central South Pacific Ocean.

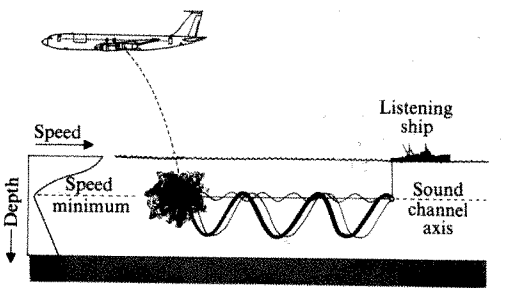


Fig. 1 Diagram of typical SOFAR propagation experiment.

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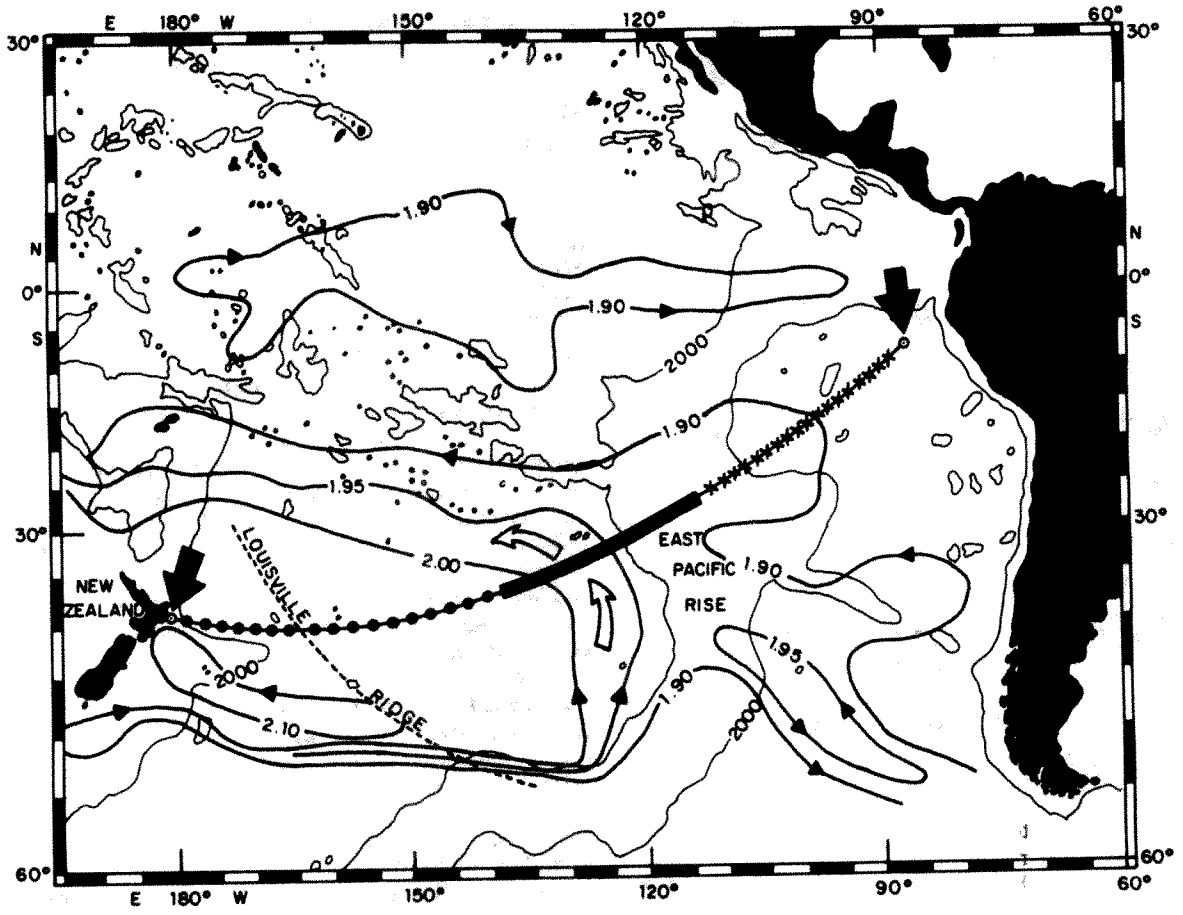


Fig. 2 Experimental track of Project Kiwi One. ⊙ designates receiver locations. Regions: *, eastern; ——, central; western. Dynamic contours (see ref. 15) indicate flow of AIW through central region (shown by arrows).

Although the South Pacific is the world's largest ocean, relatively little is known of its oceanographic and acoustic characteristics. Recent oceanographic cross-sections[6] indicate that there is a strong influx of Antarctic intermediate water (AIW) into the South Pacific from the polar regions as part of the principal anticyclonic gyre. Results from an acoustic experiment conducted in the Tasman Sea[7] indicate that such an influx would increase the attenuation of low frequency sound.

An ultra-long range sound transmission experiment to determine the acoustic properties and oceanographic regimes across the entire width of the South Pacific was jointly planned by US and New Zealand laboratories and designated as Project Kiwi One[8].

In a SOFAR propagation experiment (Fig. 1) both source and receiver are located near the axis of the deep ocean sound channel, which corresponds to a minimum sound speed. A cone of sound rays follows totally refracted paths along this axis, being only attenuated by the properties of the various water masses encountered.

The track of the Kiwi One experiment (Fig. 2) extended along a great circle between the North Island of New Zealand and the coast of South America near Peru, a distance of 10,000 km. Hydrophones to receive acoustic signals were located at each end of the track. Underwater sound signals (small explosive sources which detonated at the nominal depth of the sound channel axis, 1,100 m[9]) were dropped from an aircraft at 50-km intervals along the path. Flying such a total distance non-stop was a formidable task, achieved in ~16 h by a modified Boeing 707 aircraft with inflight refuelling[10]. The signals received from each sound source were recorded on magnetic tape at both receiving sites and later energy analysed through 1/3 octave filter bands with centre frequencies from 32 to 250 Hz.

Typical results are shown in Fig. 3 as a function of range for centre frequencies of 32, 64, 125 and 250 Hz. The range in this case is shown from the New Zealand receiver; similar results were obtained from the South American end. The transmission loss has been corrected for spreading loss, hence, the slope of a line fitted by a least squares analysis through the data points will give the rate at which sound is attenuated[11]. Three distinct changes in attenuation loss occur, designated as the western, central and eastern regions, each a distance of ~3,000 km. The highest attenuation is in the central region.

A plot of the resulting attenuation coefficients as a function of frequency (Fig. 4) shows that at the higher frequencies all regions approach the predicted regional values of attenuation for the South Pacific based on *p*H dependent absorption[12]. At

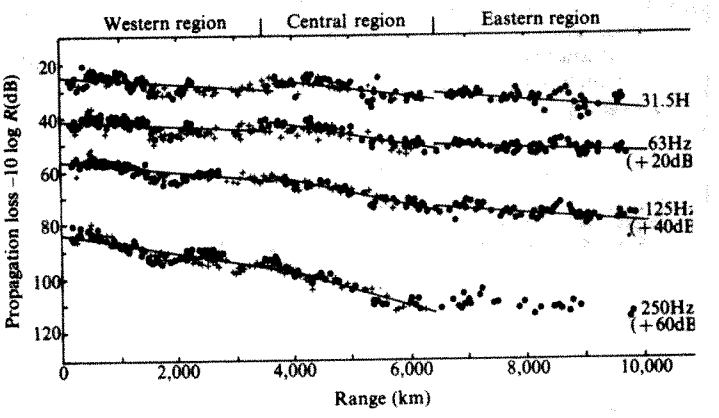


Fig. 3 Propagation loss (corrected for spreading loss) values obtained at New Zealand receiver for 1/3 octave filter bands. Levels shifted for clarity. Range is from New Zealand receiver. Fitted lines, by least square analysis, determine attenuation coefficients for each region. +, Eastbound; ●, westbound.

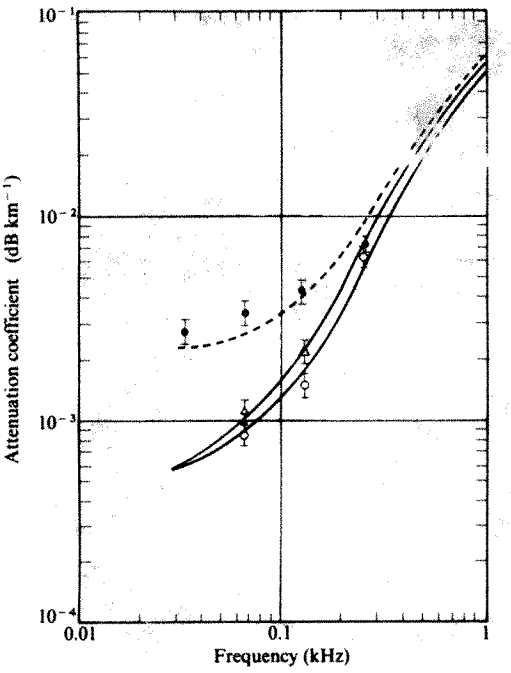


Fig. 4 Attenuation values measured in eastern region ○, central region ●, western region △ compared to attenuation obtained in Antarctic intermediate water (– – –), and subtropical water (——) in ref. 7. Upper solid line includes absorption component for western region, lower solid line includes absorption component for eastern region, based on ref. 12. Error bars indicate 90% confidence limits.

the lower frequencies, where it is hypothesised that the principal attenuation mechanism is scattering by oceanographic inhomogeneities[13], a significant difference occurs.

A comparison with water mass attenuation data obtained in the South Tasman Sea, which are similarly influenced by the Antarctic circumpolar current, shows good agreement and indicates that the eastern and western regions have values of attenuation characteristic of subtropical water masses while the central region has a higher attenuation characteristic of AIW. The higher attenuation in AIW has been attributed by Kibblewhite[14] to greater temperature inhomogeneities in this water mass. This influx of AIW into the central region at a depth near the sound channel axis is consistent with the Scorpio[6] data and the dynamic flow suggested by Johnson[15].

The acoustic data from a complete cross-section of the South Pacific Ocean shows three distinct regions of transmission loss, the highest occurring in the central region. The differences are due to changes in the value of low frequency attenuation. The results obtained were consistent with other attenuation measurements in Southern Hemisphere water masses and indicates that the higher attenuation in the central region is due to an influx of AIW.

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Alanine enantiomeric ratio in the combined amino acid fraction in seawater

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In deep ocean waters the concentrations of free amino acids are very close to blank levels, while surface waters contain only very low quantities[1,2]. However, the concentration of 'combined' amino acids (whoch occur at all ocean depths in significant quantities) is roughly 10 times that of free amino acids[1,2]. These combined amino acids are bound in some sort of polymeric material and are released only on hydrolysis in strong acid. The combined amino acid material apparently has a substantial D-aspartic acid content; the D/L aspartic acid ratio in the combined amino acid fraction, isolated from water from the Sargasso Sea, is ~0.4 at all depths[2]. It has been suggested that combined amino acids were not actually part of the dissolved organic material in the oceans but were components of bacteria that passed through the filter used in the initial seawater-processing step[2,3]. Although in most organisms only the L-enantiomers of the amino acids are present, becateria contain several D-amino acids in their cell-wall proteins[4]. To verify the bacterial explanation we have carried out investigations into the enantiomeric composition of amino acids, besides aspartic acid, in the combined fraction. We measured the alanine enantiomeric ratio in seawater samples from three stations in the Pacific Ocean. Alanine was chosen because it is one of the more abundant components of the combined amino acid fraction in the oceans[1,2], and because D-alanine is a major component of bacterial cell-wall proteins[4].

The combined amino acids were isolated from the seawater samples using the procedures described elsewhere[1,2]. The D/L alanine measurements were made by gas chromatography, using the *N*-trifluoroacetyl-L-propyl peptide methyl ester method[5]. Some of the samples were sent to Dr K. Kvenvolden (US Geological Survey, Menlo Park, California) for independent analyses. The results in Fig. 1 show that the D/L alanine ratio has a value of around 0.5 in surface waters and increases to ~0.8–1.0 in deep water at all three stations. The ratios of the other amino acids measured were in the range of ~0.1–0.2 at all stations and depths. Also, the D/L ratios obtained at Scripps are compatible with those determined by Kvenvolden's laboratory.

If the combined amino acids in the oceans were simply bacterial components, then the results in Fig. 1 indicate that in deep waters, the bacterial proteins have a D/L alanine ratio close to 1.0. We feel it is unlikely, however, that bacteria would incorporate D- and L-alanine in a proportion close to 1.0, which is the chemical equilibrium ratio for the enantiomers. Also, deep water has been sampled at widely different locations and the D/L alanine ratio never exceeds 1.0. The various water samples might contain different bacterial species that would have differing D/L alanine ratios in their cell-wall proteins, and that they might have D/L alanine ratios both less and greater than 1.0. The fact that the D/L ratios do not exceed 1.0 suggests that we are observing the product of a chemical process, not a bacterial cell-wall component.

One explanation for the high D/L alanine ratio in the combined amino acids in deep oceanic waters is that racemisation has taken place. Racemisation has been observed in a variety of fossil materials and in deep-sea sediments (see ref. 6 for review). However, estimates of the racemisation rate in the ocean[2,7] indicate that it would require hundreds of thousands of years to produce a D/L alanine ratio of ~1.0; this is much longer than the mixing times of the oceans (see ref. 8). Alanine may, however, be a component of marine humus. As this material is

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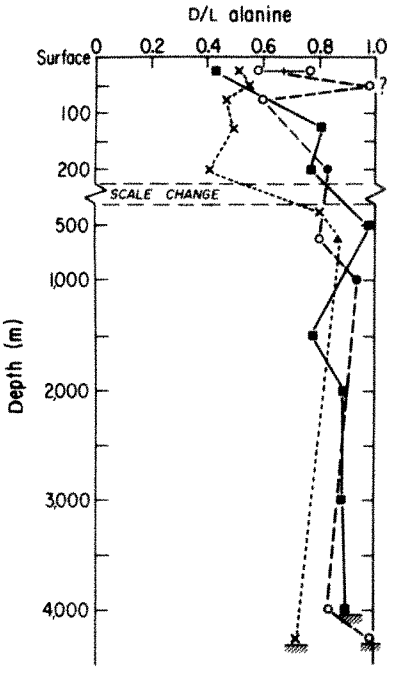


Fig. 1 The results of the D/L alanine measurements for stations: *a*, the North Pacific gyre (28°33.5′ N, 155°30.7′ W) (○); *b*, ~670 km south-west of San Diego (28°25.0′ N, 122°31.0′ W) (×); and *c*, ~60 Km off Peru (15°22.0′ S, 76°02.3′ W) (■). ● and ▲ are independent measuements carried out by Dr K. Kvenvolden and D. Blunt, US Geological Survey.

apparently highly unreactive and of considerable age[3], racemisation might have occurred during the time that the marine humus remains in the oceans. The extent of racemisation of the various amino acids in the combined amino acid fraction seems to discount this possibility. The relative rates of the amino acid racemisation reactions[6] indicate that aspartic acid would be more rapidly racemised than alanine, while glutamic acid should have a rate similar to alanine. This is not what we found for combined amino acids in the ocean; alanine was found to be the most highly racemised fo all the amino acids.

An interesting explanation for the high D/L alanine ratios in deep water and for the increase in the D/L alanine ratio with depth in the ocean involves a dehydration reaction of serine; this reaction produces alanine that is racemic (D/L ratio of 1.0). Serine dehydration has recently been found to occur in foraminiferal tests in deep sea sediments[9] and in fossil molluscs[10]. Several observations indicate that serine dehydration accounts for the high D/L alanine ratios in deep ocean waters. Our analyses indicate that serine is one of the more abundant components of the combined amino acids in surface waters, but a relatively minor component in deep waters, suggesting that this amino acid is fairly unstable and has decomposed. Whereas the serine content of the combined amino acid fraction decreases with depth, the alanine content increases; these observations are consistent with the serine dehydration pathway.

Threonine can also undergo dehydration via a pathway identical to that of serine, producing (in this case) the amino acid α-amino-N-butyric acid (ABA); the ABA produced by this mechanism is racemic[9]. ABA is a non-biological amino acid and is apparently not produced by any pathway other than threonine dehydration. The presence of ABA in seawater therefore would be convincing evidence that the serine and threonine dehydration reactions occur in the ocean. A peak that had a retention time similar to ABA was present on the amino acid analyser chromatograms of all of the deep water samples. The surface water samples seem to contain a trace quantity of ABA; the relative amount of ABA increased in the deep water samples relative to that in surface waters. To confirm that ABA was indeed present in deep oceanic waters, we analysed a 20-l water sample collected from a depth of 2,800 m at a station ~670 km off the coast of southern California. We collected the fraction in which the ABA peak eluted from the amino acid analyser column. This fraction was then desalted and analysed by gas chromatography. (The procedures used for the ABA collection and the subsequent analysis are identical to those described elsewhere[9].) The gas chromatographic analysis indicated the presence of two peaks which had retention times identical to those of the D and L enantiomers of ABA. Moreover, the gas chromatographic results indicate that the D/L ABA ratio was ~1.0, in accord with that predicted from the threonine dehydration pathway.

These various results show that the dehydration reactions of serine and threonine are quite possibly taking place in the oceans; this therefore provides some of the first experimental evidence that chemical diagenesis of dissolved organic material occurs in the sea. Alteration reactions, such as serine and threonine dehydration, may cause the organic material in the ocean to be less biologically useful, and thus they could partially explain why the bulk of the dissolved organic carbon in the oceans is of considerable age[3]. Nothing is known about the rate of the serine and threonine dehydration reactions in the oceans. Future investigation of the kinetics of these reactions should provide important new insights into the reactivity of the dissolved organic material in the sea.

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The surface charge of suspended particles in estuarine and coastal waters

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The colloidal stability of particles transported in suspension by rivers is controlled by surface electrical properties[1] which are conventionally regarded as undergoing rapid change during estuarine mixing[1–3] by interaction with polyvalent ions, especially Ca^{2+} and Mg^{2+}. Although wide differences in rates of coagulation of clay minerals as a function of salinity have been demonstrated in the laboratory[4,5], Gibbs[6] and others[7,8] have concluded that field studies provide no real evidence for the importance of such differential flocculation. Instead, changes in clay mineral distribution with distance from the source river can be accounted for by differences in the particle size spectra and the resulting sedimentation velocities. The effects of differing electrical and surface properties on coagulation rates are apparently suppressed by the formation of uniform films of metal oxides and/or organic matter around the particles[6]. We present here direct measurements of the electrokinetic charge on river and estuarine particles which confirm this hypothesis.

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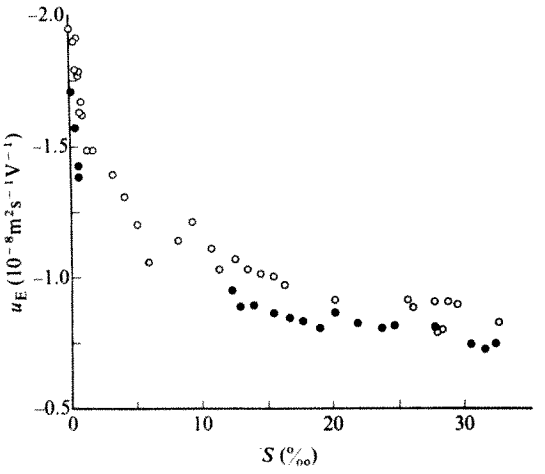


Fig. 1 Electrophoretic mobility (u_E) of particles collected in the Conwy estuary as a function of salinity (S). ●, 29 April 1979 survey; ○, 17 June 1979 survey. The electrophoretic mobility is the velocity per unit of applied electric field of particles moving at the stationary levels of the cell. Field strength was calculated from the current passed at the time of measurement and the electrolyte conductivity, determined separately. Each point plotted is the mean of determinations on at least 10 particles at each of the two stationary levels. In addition, measurements were made for all particles in each direction by reversing the field polarity. The observed standard deviation of the values was typically 14% with most in the range 6–20%. This implies a standard error in mean u_E values plotted of typically 2–4% which was confirmed by repetitive measurement of a single sample.

River and estuarine samples (20–35 per survey) have been collected from the Alde (Suffolk, 8 February and 19 March 1979), Orwell (Suffolk, 16 April 1979), Beaulieu (Hampshire, 17 May and 4 June 1979) and Conwy (North Wales, 29 April and 17 June 1979). For each sample the salinity of the water and the electrophoretic mobility of the suspended particles were measured within about a week of collection; numerous prior tests showed that storage (at 6 °C in the dark) for this period led to no significant changes in electrophoretic mobility[9]. Mobilities were determined with a Rank Mark II particle electrophoresis apparatus using a rectangular quartz cell[9]. Full details of the procedure, which utilised Ag/AgCl electrodes and a miniature measurement cell to allow determinations to be made in full strength seawater, will be published elsewhere. Samples were analysed for dissolved organic carbon (DOC) and surfactant activity in the water. The results of these analyses and the method developed for the measurement of surfactant activity will be reported separately. River waters flowing into the estuaries were analysed for their major dissolved cationic components (Na^+, K^+, Mg^{2+}, Ca^{2+}).

Representative plots for the relationship between electrophoretic mobility (u_E) and salinity (S) are shown in Figs 1 (Conwy) and 2 (Alde). The results for the Beaulieu are very similar to those for the Conwy. Those for the Orwell closely resemble the results given for the Alde. A composite plot giving the combined data for all seven surveys is shown in Fig. 3. The electrophoretic mobility is the appropriate parameter to be used in discussing changes in electrokinetic behaviour, because it is the only quantity which can be measured directly in this type of experiment[10]. Its relationship to the electrokinetic or ζ potential and the electrokinetic charge density is theoretical and depends in a complex manner on particle radius of curvature and electrolyte concentration[11].

Figures 1–3 clearly show that the particles are negatively charged at all salinities. The negative charge at high salinity is in agreement with previous electrophoretic measurements made directly on suspended particles in seawater[9,12]. Figure 4 shows the results of electrophoretic measurements made on 160 individual particles in a sample of North Sea water, which confirm the absence of positively charged surfaces. In contrast to these findings, streaming current measurements made on coarse-grained sediment particles in seawater (natural or synthetic)/distilled water mixtures indicate a change from negative to positive ζ potential at salinities which range from 1.8 to >20‰ (refs 2, 3). This discrepancy may result from differences in the sizes of particles examined by each method or in the nature of the particulate material itself. In addition, the indirect nature of the streaming current measurements may have introduced significant changes through storage effects, chemical pretreatment of the sediments, lack of equilibration with the measurement electrolytes and (in some cases) the use of synthetic media. These factors will be of particular importance if natural surface coatings are removed or altered.

It is often assumed that flocculation of riverborne particulates occurs when their charge is neutralised on mixing with high ionic strength seawater in the estuarine zone[1]. The fact that all the particles examined in the present study have a substantial negative charge means that this mechanism of flocculation in estuaries is probably not important.

In the Alde and Orwell estuaries there is a gradual and almost linear decrease in negative mobility with increase in salinity. For the Conwy and Beaulieu estuaries a similar decrease occurs in the mid to upper salinity regions, but at low salinities u_E changes more dramatically. This division of the estuaries studied into two types (Figs 1 and 2) is almost certainly due to differences in river water composition. Both the Orwell and Alde rivers contain relatively high levels of dissolved cations, especially Ca^{2+}, whereas the Rivers Conwy and Beaulieu have substantially less dissolved salts. This means that in the calcareous rivers some of the natural electrokinetic charge of the particles will already be neutralised by incorporation of cations (particularly polyvalent ones) into the fixed part of the double layer, whereas for more acidic rivers, such as the Beaulieu and Conwy, this effect will not occur until the low salinity part of the estuarine zone. Support for this argument comes from laboratory experiments in which suspensions of seawater particles were mixed with high and low Ca^{2+} river waters and distilled water. Irrespective of the origin of the marine particulates, the high Ca^{2+} river water gave a u_E/S plot resembling Fig. 2, whereas low Ca^{2+} river water, and more especially distilled water, produced plots of similar shape to Fig. 1.

Apart from differences due to river water composition, the combined data in Fig. 3 show remarkably little dispersion and there is also good agreement between the results of independent surveys of the same estuary (Figs 1 and 2). Perhaps more importantly, the variations in u_E of individual samples are small (see legend to Fig. 1) indicating a high degree of surface uniformity. The results shown in Fig. 4 are typical in this respect; at

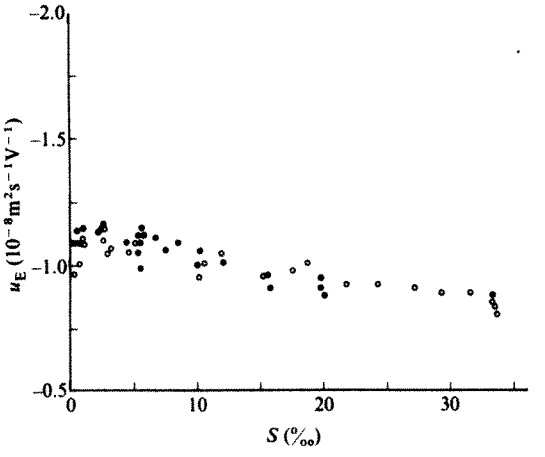


Fig. 2 Electrophoretic mobility (u_E) of particles collected in the Alde estuary as a function of salinity (S). ●, 8 February 1979 survey; ○, 19 March 1979 survey. Standard errors of mean u_E values are comparable to those of the Conwy estuary (Fig. 1).

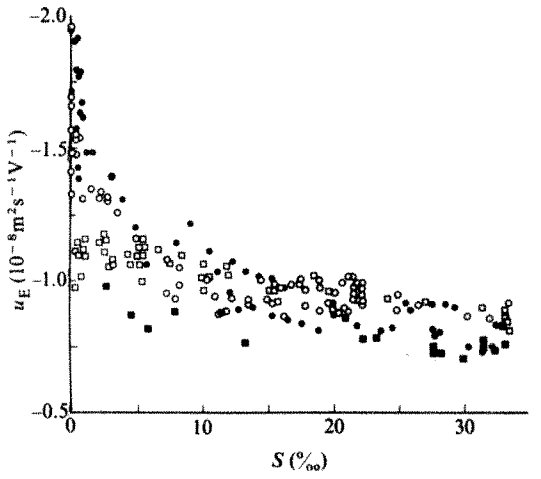


Fig. 3. Composite plot showing electrophoretic mobility (u_E) of particles as a function of salinity (S) for the four rivers studied. ○, Beaulieu estuary; ●, Conwy; □, Alde, ■, Orwell. Data for the two separate surveys of each of the first three estuaries have been combined in the one symbol.

least half of the total u_E variability (13%) in this case is attributable to experimental errors (mainly due to focusing)[13]. In view of the highly varied nature of the particle types involved (various clay minerals, aluminosilicates, quartz grains, calcite, living and dead organisms, detritus), the present results provide the first direct evidence of a dominant role of oxide and/or organic films in determining the surface properties of suspended estuarine particles. Previous work using similar methods has established that natural organic surfactants largely control the properties of surfaces introduced into seawater through the formation of cohesive films[9,12,14,15]. Surfactant activity and DOC results for the present surveys indicate levels of adsorbable organic matter in excess of those in seawater. Accordingly, in these estuarine systems organic coatings are expected to be at least as important as in seawater. With respect to control by oxide films, the Beaulieu estuary (Fig. 3) is an area of rapid removal of dissolved iron[16] and there is some evidence for uptake of the precipitated material onto particle surfaces[17].

As surface active organic matter is ubiquitous in natural waters, it seems reasonable to conclude that the basic principles revealed here are sufficiently general to apply in other areas. For example, the uniformity of electrokinetic charge on suspended estuarine particulates, arising from the dominant presence of surface coatings, is able to explain the apparent lack of evidence for differential flocculation of minerals in field studies of sediment distribution[6–8]. Furthermore, it seems likely that surface films will exercise a major role in chemical transformations involving particulate matter. Thus, for example, uptake/release of dissolved trace metals by particles may well depend on the chemistry of the organic matter forming the film rather than that of the underlying solid matrix.

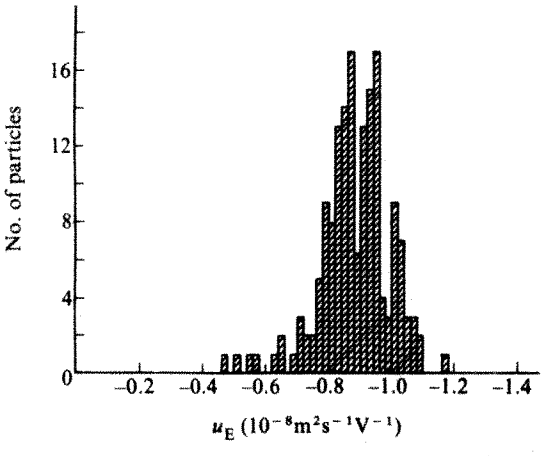


Fig. 4 Frequency histogram of electrophoretic mobility (u_E) of 160 natural suspended particles in coastal North Sea waters collected 6–7 km south-east of Great Yarmouth, Norfolk. Combined data for both stationary levels. Mean and standard deviation of u_E; $0.89 \pm 0.12 \times 10^{-8}$ m^2 s^{-1} V^{-1}.

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Helium and xenon in deep-sea basalts as a measure of magmatic differentiation

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There has been much recent interest in the rare gas abundances of terrestrial materials, due mainly to the discovery of 'excess' radiogenic Ar and He trapped in deep-sea glasses[1–4]. The abundances of these gases indicated that such materials might effectively trap the internal Earth's atmosphere, and evidence of trapped primordial gases has by now been found not only in deep-sea glasses[5–10], but also in xenolithic inclusions[11–15], deep ocean waters[16], CO_2 well gas[17–19], volcanic gas[20] and diamonds[21]. The rare gas elemental abundances measured in these samples may not quantitatively reflect the deep-Earth pattern. Several elemental fractionation processes have been suggested, ranging from selective enrichment of the lighter gases in an upward-rising magma[22,23] to posteruptive diffusion loss which would selectively deplete the lighter gases[24]. Each of these processes will affect concentrations of the helium and xenon isotopes in a characteristic manner, thus leaving in the glasses an isotopic fingerprint of what has occurred. New data are reported here which indicate extremely small concentrations of fissiogenic xenon in deep-sea basalts containing large concentrations of trapped excess radiogenic helium and argon. The resulting high $^4He_r/^{136}Xe_f$ ratios are diagnostic of fractionation during the magmatic processes leading to eruption rather than of posteruptive loss of gas (which would show the opposite effect). The results indicate that studies of volatiles in general and of the rare gases in particular in such mantle-derived samples, even in those with no subsequent alteration or contamination, may be profoundly influenced by mass-dependent pre-eruptive effects. The consequences of this fractionation for the deep-Earth K/U ratio are discussed.

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Table 1 Rare gas data for tholeiitic glasses from the Vema Fracture Zone (MAR)

Sample	^4He (10^{-8} scc g^{-1})	$^4He/^{40}Ar_r$	Xe (10^{-12} scc g^{-1})	$^{129}Xe/^{132}Xe$	$^{131}Xe/^{132}Xe$	$^{134}Xe/^{132}Xe$	$^{136}Xe/^{132}Xe$	$^{136}Xe_f$ (10^{-15} scc g^{-1})	$^4He/^{136}Xe_f$ ($\times 10^9$)
AT-72 (1)	1,500±300	18±2	1.4	0.980±0.01	0.788±0.01	0.388±0.007	0.343±0.006	4.8	3
(2)	—	—	13	1.00 ±0.01	0.809±0.01	0.386±0.006	0.330±0.005	<6	—
(4)	860	16	1.0	1.02 ±0.01	0.768±0.01	0.390±0.007	0.355±0.006	2.6	3.3
(7)	—	—	3.5	0.968±0.01	0.796±0.01	0.389±0.007	0.337±0.006	6.6	—
(8)	1,150[24]	15[24]	—	—	—	—	—	—	—
(9)	1,300[24]	17[24]	—	—	—	—	—	—	—
Avg.	1,200±270	16±1	—	—	—	—	—	4.6±2	2.4±2
AT-88 (1)	1,800±600	9±3	1.6	1.07 ±0.03	0.807±0.02	0.40±0.01	0.342±0.005	5.2	3.5
(2)	2,300	7.2	62	0.978±0.02	0.758±0.02	0.381±0.008	0.328±0.006	—	—
(3)	2,400[24]	8.3[24]	—	—	—	—	—	—	—
(4)	2,400[24]	7.3[24]	—	—	—	—	—	—	—
AT-92 (1)	2,400	9.3	20	0.986±0.01	0.780±0.01	0.374±0.01	0.330±0.005	—	>1
(2)	1,900	—	4.6	1.01 ±0.02	0.778±0.01	0.390±0.005	0.332±0.006	<8	>2
(3)	1,600[24]	6.8[24]	—	—	—	—	—	—	—
(4)	2,370[24]	12[24]	—	—	—	—	—	—	—

Absolute accuracy is ±15% for Ar, ±20% for He and Xe.

Most terrestrial ^4He is due to the radioactive decay of the U and Th families. A small contribution to terrestrial Xe is due to the spontaneous fission of U. The Th/U ratio of non-sedimentary, non-metamorphic terrestrial rocks is 3 ± 1. The production ratio of radiogenic He(^4He_r) and fissiogenic Xe($^{136}Xe_f$) is nearly constant with time and with the nearly constant Th/U ratio ($^4He_r/^{136}Xe_f = 4.2\times10^8 \pm 10\%$). Finally, He and Xe are the lightest and heaviest of the rare gases, respectively, and so their separation by any mass-dependent process will show the largest possible effects. Therefore a $^4He_r/^{136}Xe_f$ ratio $\sim 4.2\times10^8$ in a given material is evidence that no significant mass fractionation effects have disturbed the rare gas pattern, a value $<4.2\times10^8$ is evidence for preferential loss of He, and a value $>4.2\times10^8$ is evidence for preferential He enrichment.

Samples of glassy basalts outcropping in the Vema Fracture Zone (Mid-Atlantic Ridge), previously shown to have high $^4He_r/^{40}Ar_r$ ratios characteristic of efficient trapping of the pre-eruptive rare gases[24], were selected for analysis. The samples were melted and partially vaporised in an r.f.-induction furnace, and the resulting gases were purified over two successive Ti-getters at various temperatures. Helium was measured while Ar and Xe were adsorbed on charcoal; the Ar and Xe were then successively released and measured. The instrument was a Reynolds-type steel 4.5″–60° mass spectrometer. Isotopic ratios were determined by extrapolation of several spectra sweeps back to entrance time; absolute amounts were calibrated by pure gas standards of atmospheric (except for He) isotopic composition run alternatively with the samples; blank runs were interspersed. A correction for instrumental mass fractionation was made on the basis of the standard gases. The results are shown in Table 1; the Xe isotopic data are plotted in Fig. 1. The linear relationship follows from:

$$^{136}Xe_t = {}^{136}Xe_f + {}^{136}Xe_m + {}^{136}Xe_a$$
$$= {}^{136}Xe_f + a({}^{132}Xe_m + {}^{132}Xe_a)$$
$$= {}^{136}Xe_f + a\,{}^{132}Xe_t$$
$$\frac{^{136}Xe_t}{^{132}Xe_t} = \frac{^{136}Xe_f}{^{132}Xe_t} + a$$

where the subscripts f, m, a, and t identify the fissiogenic, mantle, atmospheric, and total components respectively, and the a represents the $(^{136}Xe/^{132}Xe)_a$ ratio. The ratio $(^{136}Xe/^{132}Xe)_m$ is assumed identical to a; but if it were identical to that of the planetary primordial component observed in primitive meteorites the error is only 3% and does not affect the conclusion. A least squares fit to the data of Fig. 1 gives a straight line with coefficient of determination $r = 0.97$ and intercept 0.329, identical within limits of error with a. The data therefore indicate mixing of a constant fissiogenic component with varying amounts of atmospheric xenon. The slope of the line gives the concentration of $^{136}Xe_f \sim 6\times10^{-15}$ scc g^{-1} (scc, standard cubic centimetre). $^{136}Xe_f$ can also be calculated for the individual samples; these values are shown in Table 1.

The He in these samples is overwhelmingly radiogenic. The primordial $^4He/^3He$ ratio measured in gas-rich meteorites is ~3,500. ^3He has not been measured in the samples considered here, but the highest concentration measured in deep-sea glasses is about 90×10^{-12} scc g^{-1} (refs 8, 10), and therefore $^4He_p < 30\times10^{-8}$ scc g^{-1}, compared to the total measured values of $1{,}000$–$2{,}000\times10^{-8}$.

The resulting $^4He_r/^{136}Xe_f$ ratios are $>2\times10^9$ for all three glasses (Table 1). This is nearly an order of magnitude greater than the production ratio, supporting Alexander's suggestion of mass fractionation due to preferential upward migration of the lighter elements in the rising magma[22,23].

In this argument I have attributed the excess ^{136}Xe to spontaneous fission of ^{238}U. All the isotopes except ^{136}Xe are within limits of error of the atmospheric abundances, leading naturally to the supposition that it is something particular about ^{136}Xe that leads to its enrichment; yet the limits of error do not preclude some contribution from an alternative mechanism of isotopic enrichment of the heaviest xenon isotope, such as a mass fractionation process. Nevertheless, my conclusions remain valid despite any such possible complications. The low concentration of 'fissiogenic' xenon gives a high $^4He_r/^{136}Xe_f$ ratio, which indicates ^4He enrichment by fractionation of the lighter gas. If part of the excess ^{136}Xe attributed to a fissiogenic origin is actually due to mass fractionation (or to any other process) then the true concentration of fissiogenic xenon is correspondingly lower, the $^4He_r/^{136}Xe_f$ ratio correspondingly higher, and this only strengthens the argument that the light gases are enriched by the fractionation process. In turn this conclusion make the possiblity of the xenon being fractionated in such a manner as to enrich the heavier isotopes unlikely, thus leading back to the

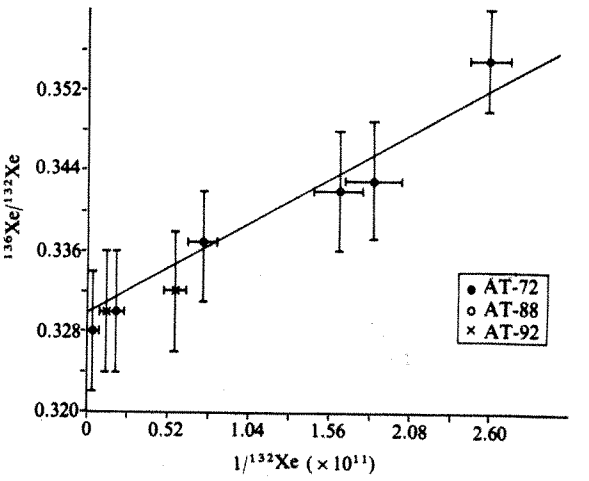


Fig. 1 Xenon isotopic ratios plotted against $1/^{132}Xe$ for the glasses.

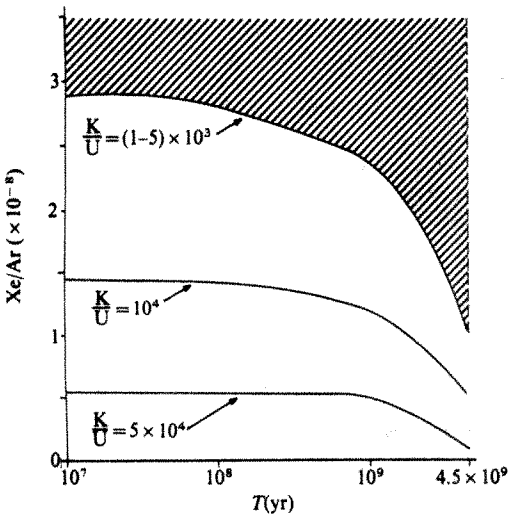


Fig. 2 The $^{136}Xe_f/^{40}Ar_r$ production ratio as a function of time.

conclusion that the excess ^{136}Xe is indeed fissiogenic. Therefore even if part of the measured excess ^{136}Xe is not fissiogenic, or if the magnitude of the excess has been overestimated, the conclusion that elemental fractionation of the rare gases has occurred remains valid; that is, the lighter-mass gases are preferentially enriched in these samples.

Previous arguments[24,25] assessing the terrestrial degassing history and/or the K/U ratio in the basalt source magma, based on the high $^4He_r/^{40}Ar_r$ ratio in these rocks, are now seen to be invalid as the He/Ar ratio must also have been enriched by this mechanism. It is not possible from these data to estimate quantitatively the effect on the He/Ar ratio, but we can use both the $^4He_r/^{40}Ar_r$ and $^{136}Xe_f/^{40}Ar_r$ ratios to set upper and lower limits to the K/U ratio. Although the results have no meaning for the present set of data, the calculation may be useful in future. The production ratio $^{136}Xe_f/^{40}Ar_r$ is shown in Fig. 2, the production ratio $^4He_r/^{40}Ar_r$ has the same form (see Fig. 1 of ref. 24). The age dependency can be eliminated by considering the ratio $^{136}Xe_f/U_m$. The uranium concentration of the oceanic upper mantle (U_m) has been estimated at >20 parts per 10^9 (ref. 26), giving

$$^{136}Xe_f/U_m < 3\times 10^{-7}\ \text{ssc g}^{-1}$$

and an age for accumulation of the rare gases $T < 4\times 10^8$ yr. This is an upper limit both because of the U limit and because the concentration of $^{136}Xe_f$ may have been enriched over the mantle value in these samples by the same process that led to enrichment of the He/Xe ratio. This age refers not to emplacement of the basalt on the sea floor but to accumulation of the radiogenic gases in the magma. The $^{136}Xe_f/^{40}Ar_r$ data of Table 1, compared to Fig. 2 for $T < 4\times 10^8$ yr, specify an upper limit of $K/U < 5\times 10^4$; the $^4He_r/^{40}Ar_r$ data, compared to the calculated production ratio (Fig. 1 of ref. 24) provides a lower limit of $K/U > 3\times 10^3$. These limits (which refer to the basalt source region) encompass nearly the total range of previous suggestions for the deep-Earth K/U ratio.

One other previous attempt to identify the mantle K/U ratio through rare gas data can be faulted by a similar analysis. Hennecke and Manuel measured a low $^4He_r/^{40}Ar_r$ ratio on a Hawaiian xenolith[12], concluding that this indicated generation from a K/U source of chondritic composition ($K/U \sim 5\times 10^4$). Their measured Xe displays a small excess at mass = 136 which can be identified as $4\times 10^{-15} < {}^{136}Xe_f < 24\times 10^{-15}$ scc g^{-1}; the $^4He = 35\pm 4\times 10^{-8}$ scc g^{-1}, so that $^4He_r/^{136}Xe_f < 9\times 10^7$, which indicates preferential loss of He. Therefore the low He/Ar ratio may be due to this He loss rather than to a chondritic source. On the other hand, in the Mitchell No. 7 CO_2 gas well of Harding Co., New Mexico, Zartman *et al.* found $^4He = 45\times 10^{-6}$ cm^3 per cm^3 (ref. 27), and Butler *et al.* found $^{136}Xe_f = 9\times 10^{-14}$ (ref. 17), giving $^4He_r/^{136}Xe_f = 5\times 10^8$. This result is well within experimental error of the theoretical value 4.2×10^8 and indicates no significant mass fractionation of the radiogenic gases. The measured $^4He_r/^{40}Ar_r$ ratio of 1.6 indicates a K/U source of $\sim 1\times 10^4$, but the source region for these wells probably reflects a crustal rather than a mantle environment.

Deep-sea glasses probably provide a rare gas inventory produced in a mantle environment and not greatly modified by crustal contamination. If a suite of such samples can be found with $^4He_r/^{136}Xe_f$ closer to the production ratio, useful limits to the mantle K/U ratio may yet be provided. If, on the other hand, we are able to specify the mantle K/U ratio independently, then measurements of the ratios $^4He_r/^{40}Ar_r$ and $^4He_r/^{136}Xe_f$ may provide a quantitative estimate of the magmatic mass fractionation process. The mantle components of all the rare gases (and of other volatiles) may then be realistically estimated from their measured abundances in deep-sea glasses.

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Refractory element fractionations among carbonaceous chondrite groups

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Chondritic meteorites consist of collections of grains formed in the solar nebula. Chondritic materials can often be resolved into components that formed in differing nebular conditions. Carbonaceous chondrites are often accorded special significance in planetary models, especially because abundances of most elements in the CI group are indistinguishable from those in the Sun. Abundances of refractory lithophiles are among the most useful parameters for the classification of chrondrites, because they systematically decrease through the sequence carbonaceous→ordinary→enstatite[1,2]. They are also the most general parameter linking groups of very similar chondrites into clans which consist of groups having certain common properties that may indicate formation within a narrow range of distances from the Sun[3]. We report here newly discovered differences in refractory abundances among the different groups of carbonaceous chondrites.

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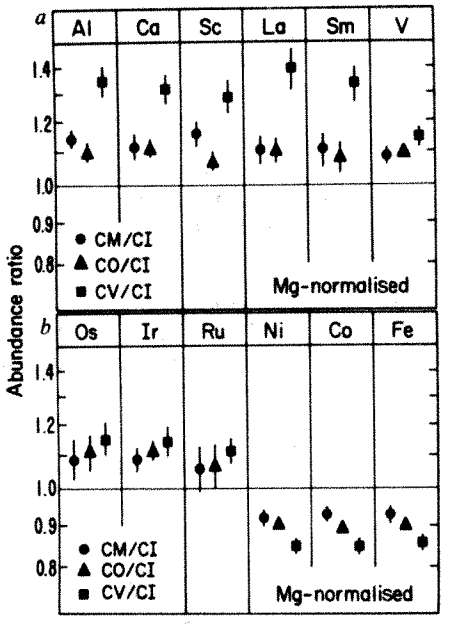


Fig. 1 Mean CI-normalised atomic abundance ratios for; *a*, refractory lithophiles; and *b*, refractory and non-volatile siderophiles. Uncertainty limits are 80% confidence limits. Our CI abundances (Mg normalised, Mg = 100) are: Al = 8.16, Ca = 5.87, Fe = 82.4, Co = 0.219, Ni = 4.48, Sc = 3.26×10^{-3}, V = 2.78×10^{-4}, La = 4.17×10^{-5}, Sm = 2.40×10^{-5}, Ru = 1.69×10^{-5}, Os = 6.34×10^{-5}, Ir = 5.49×10^{-5}.

Carbonaceous chondrites are divided into four groups, CI (high volatile abundances, no chondrules), CM (intermediate volatile abundances, small (~0.2 mm) chondrules), CO (low volatile abundances, small chondrules) and CV (low volatile abundances, large (~1 mm) chondrules). CV and CO chondrites were thought to belong to the same group, until Van Schmus[4,5] pointed out textural differences and McCarthy and Ahrens[6] found higher refractory lithophile abundances in CV than in CO. In the past it was believed that refractory lithophile abundances are indistinguishable in CI, CM and CO (refs 4, 5), and refractory siderophiles indistinguishable in all four carbonaceous groups[7,8]. In fact, our neutron activation data show that the carbonaceous groups can be divided into CI, CM–CO and CV clans based on differences in refractory lithophile abundances, and that refractory siderophiles do not parallel refractory lithophiles.

Nineteen carbonaceous chondrites were analysed: two CI chondrites (Orgueil, Alais), nine CM chondrites (Murray, Murchison, Mighei, Nogoya, Santa Cruz, Cold Bokkeveld, Yamato 74662, Cochabamba, Allan Hills A77306), five CO chondrites (Lancé, Felix, Ornans, Kainsaz, Warrenton) and three CV chondrites (Allende, Mokoia, Vigarano). Two samples (~300 mg each) of each meteorite were analysed in separate irradiations, with the exception of Orgueil (four samples), Allende (six samples), Santa Cruz (one sample) and Allan Hills A77306 (one sample). Among the elemental concentrations determined were several refractory lithophiles (Ca, Al, Sc, La, Sm), refractory siderophiles (Os, Ir, Ru) and a suitable normalising element (Mg). Complete data for some 25 elements will be published elsewhere.

The common practice of combining elemental concentration data from sources that may include systematic biases can unduly 'broaden' the abundance ranges. One such bias can result from the variable state of hydration of CI and CM chondrites. Producing our own large data base for each carbonaceous chondrite group and determining a suitable normalising element in the same sample enabled us to avoid these problems and has resulted in a clearer picture of elemental abundance variations among the different groups.

Refractory lithophile abundances in CM, CO and CV chondrites relative to CI are plotted in Fig. 1*a*. The data are Mg-normalised as we do not determine Si. Normalising to Si would not noticeably change the plots, as the Mg/Si atom ratio is essentially identical for CI, CM and CO and at most 3% higher for CV[6]. Our results confirm the observation[6] that CV refractory lithophile abundances are considerably higher (~×1.34) than those in CI. The higher abundance of refractories in CV chondrites may result from an enhancement of the large refractory inclusions present in this group relative to the other components (matrix and chondrules) carrying the major lithophiles Si and Mg. The higher abundance of refractory inclusions is attributed to differential settling of grains to the median nebular plane[9], with the rate of settling being proportional to the density and radius of the grains. At the median plane grains could agglomerate to planetesimals by gravitational collapse[9,10]. Mg concentrations in the refractory inclusions in Allende are lower than those in the whole rock; V concentrations[11,12] are higher, but inclusion/CI ratios are only about half as high as values found for highly refractory elements[12,13], thus accounting for the lower CV enhancement (×1.14) of V.

Our most unexpected discovery was that CM and CO refractory lithophile abundances are distinctly higher, by ~1.12 and ~1.09, respectively, than those in CI chondrites. Oxygen isotope data[14] are not inconsistent with an interpretation that the ~10% difference in refractory contents between CM–CO and CI indicate formation at different nebular locations. CI chondrites and CM matrix scatter along fractionation lines differing about 3% in $^{17}O/^{16}O$ ratio when extrapolated to the same $^{18}O/^{16}O$ ratio. The CI results are whole rock data and probably reflect equilibration with nebular gases. It may be that the CM data result from equilibration inside a parent body, but if they also reflect nebular equilibration, the differing O isotope compositions indicate different formation locations for CM and CI.

The rare refractory-rich inclusions in CM and CO chondrites[15–17] are smaller (~0.1 mm) than those typical of CV chondrites (~1 mm). As refractory inclusions are unknown in CI chondrites, the presence of these in CM and CO could possibly account for the small refractory lithophile enrichment. McSween[18] reports that anhydrous inclusions are about 2.5 times more abundant in CO than CM chondrites. If the refractory fraction of anhydrous materials is the same in each group and our simple model is correct, the abundance of refractory-rich materials should be about the same in CM and CO. However, CM refractory inclusions apparently have higher refractory contents than CO inclusions[17], and part of the

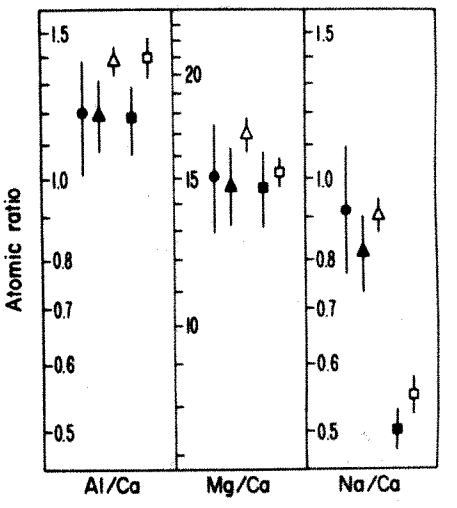


Fig. 2 Element/Ca ratios for CI and CM carbonaceous chondrites and the Sun from this work (△, CI; □, CM) and from compilations of Holweger[24] (●, solar; ▲, CI; ■ CM). Our CM Mg/Ca ratio is closer to the solar ratio than our CI value, but our Na/Ca value, like Holweger's is much lower than the reported solar ratio.

discrepancy may also have resulted from aqueous alteration and destruction of some CM inclusions[18,19].

It thus seems best to divide the carbonaceous chondrites into three separate clans: CI, CM–CO and CV chondrites. The refractory lithophile data are inconsistent with the formation of CI, CM and CO chondrites from two rigidly defined components (matrix + anhydrous minerals including chondrules)[20].

Figure 1*b* shows Mg-normalised refractory siderophile abundances for CM, CO and CV relative to CI. Also plotted are the siderophiles Fe, Ni and Co that are neither refractory or volatile. Refractory siderophiles are 1.06 to 1.15 times higher in CM, CO and CV than in CI. Previous analyses[7,8] have not revealed this enrichment, perhaps partly as a result of the lack of internal normalisation and partly by the sometimes seemingly arbitrary selection of mean CI concentrations. Morgan and Lovering[21] did note a small enhancement of Os and Re in CM relative to CI but attributed it to probable sampling errors. Refractory siderophile abundance ratios seem to decrease as CV > CO > CM. In each group Os and Ir ratios are about the same whereas Ru values are slightly smaller. This may be related to the fact that Ru is less refractory[22]. Relative to Os and Ir, CI-normalised Ru is less abundant in one refractory inclusion[13] and in 50 metal particles from another inclusion[23] of the Allende CV chondrite. Excluding Ru, abundance ratios of the refractory siderophiles in CM and CO chondrites are approximately the same as those of refractory lithophiles within analytical error, whereas in CV refractory siderophiles are about 15% lower than refractory lithophiles.

Refractory siderophiles are on average less abundant than refractory lithophiles in type B and coarse-grained refractory inclusions from Allende[24], and the siderophile/lithophile ratio is still lower in fine-grained inclusions. This suggests why refractory siderophiles are not as enriched as refractory lithophiles in CV chondrites. If a significant fraction of the refractory siderophiles was present in other kinds of carrier grains, these grains may also have been the dominant carriers of the common siderophiles (Fe, Co, Ni) that are nearly 15% deficient in CV relative to CI (Fig. 1*b*). We recently analysed a fine-grained chondritic inclusion from Allende[25] and found it to be enriched in refractory and common siderophiles, but depleted in refractory lithophiles relative to CV chondrites. The inefficient settling to the nebular midplane of similar fine-grained material could account for the observed siderophile/lithophile fractionation at the CV formation location.

On the other hand, the lower abundance ratios of Fe, Ni and Co in CM (×0.93) and CO (×0.90) relative to CI is not paralleled by a resolvable decrease in the abundance of refractory siderophiles relative to refractory lithophiles, suggesting that the carrier of the lost Fe, Ni and Co in CM and CO chondrites must have had much lower refractory siderophile/Ni ratios than the siderophile carrier lost during the formation of CV chondrites. Thus the carriers of both refractory siderophiles and refractory lithophiles were different at the CV location from those at the CM–CO location.

The actual nebular processes were probably more complex than those outlined here. Instead of two (one refractory, one nonrefractory) lithophile and two siderophile components there was probably a continuum of compositions which we have roughly approximated by a four-component model. It would seem that the four groups of carbonaceous chondrites are not as closely and as simply related as earlier thought, but are distinguished by chemical and isotopic fractionations suggesting their formation in differing conditions, and probably at differing distances from the Sun.

Recently, Holweger[26] examined the compositional relationship between the solar photosphere and chondrites to determine which chondrite group best approximated the solar composition. His work included five elements (Na, Mg, Al, S, Ca) determined to precisions of about ±10% in the Sun. Because of our improved data base, and because some chondrites were incorrectly classified in Holweger's data source, it seemed worthwhile to repeat the comparison. We do not analyse for S, so it is not included. In Fig. 2 Holweger's CI, CM and solar ratios of Al, Mg and Na to Ca are compared with our chondrite data. Our Al/Ca ratios are higher than Holweger's, but agree well with X-ray fluorescence data[6]. Holweger's conclusion that CI chondrites closely resemble the solar composition is also supported by our data. All CI, CM and solar ratios for non-volatiles agree within experimental error, but the CM ratio for the volatile Na is almost half the reported solar ratio. However, some other chondrite groups have higher Na abundances approaching CI values, and accurate solar data on additional volatile elements are needed before the CI–solar congruence can be verified.

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Radiocarbon dating evidence on the impact of atmospheric pollution on upland peats

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When radiocarbon dating is applied to young sample materials, the results are subject to the Suess effect[1]: that is, the concentration of atmospheric ^{14}C has been diluted by carbon dioxide containing negligible ^{14}C from the burning of fossil fuels since the onset of the industrial revolution. As this carbon dioxide is incorporated into growing plants in the normal manner, radiocarbon dating of young plant material tends to produce spuriously old dates. The age discrepancy could, in theory, be as much as 250 yr (ref. 2). However, during a study into the age, origin and vegetational history of upland blanket peats in South Wales, substantially older dates were obtained from recent horizons. The dating of different peat fractions by radiocarbon assay suggested that the substantially older dates derived from contamination by particulate pollution during the industrial revolution. The scale and impact of this pollution are discussed in the context of these results.

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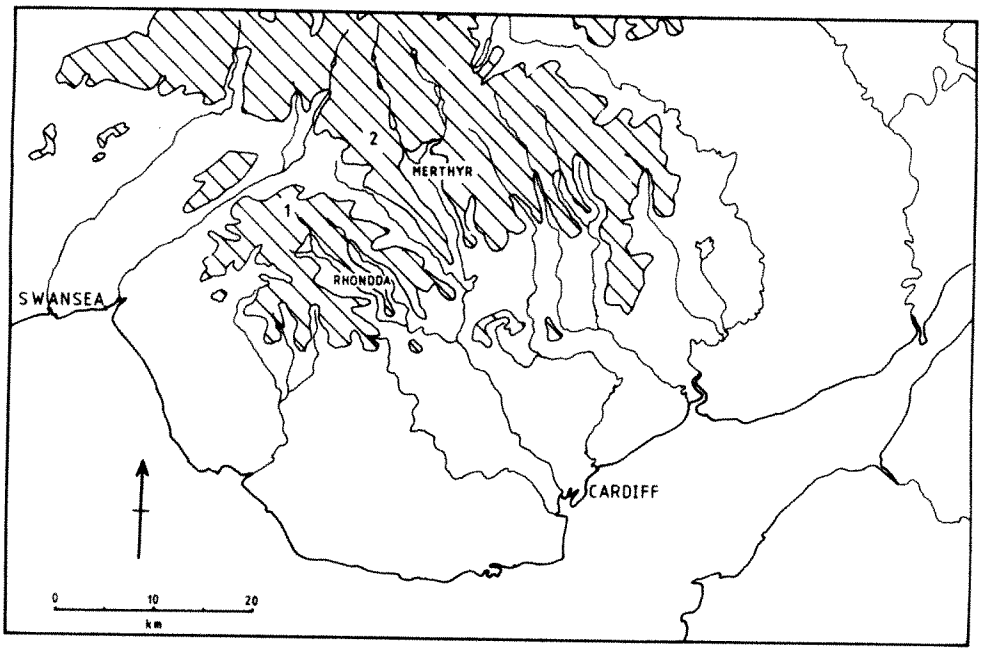


Fig. 1 Location of sites on map of South Wales: 1, Cefn Ffordd; 2, Coed Taf. Land over 300 m OD shown.

The blanket peats of upland South Wales are widespread and often shallow. Two profiles with less than 40 cm peat accumulation were investigated by pollen analysis, the sites being in close proximity to the industrial Rhondda and Merthyr valleys: Cefn Ffordd (SN 906032, 600 m OD) and Coed Taf (SN 988108, ~400 m OD) (see Fig. 1). In pollen diagrams from both sites, pollen spectra in the upper horizons showed a distinct change from those characterised by high Ericaceae values (mainly *Calluna vulgaris*—heather or ling) to a pronounced dominance of Gramineae (grass) pollen (Fig. 2). This seemed to record a shift from Callunetum to the present vegetation in which *Molinia caerulea* (purple moor grass) is dominant (Cefn Ffordd s now afforested with spruce). To date this and other changes, peat samples were prepared for radiocarbon dating using a pretreatment designed to remove those components likely to give an underestimate of the true radiocarbon age of the peat. The potentially more mobile 'humic acid' fraction was extracted in two alkali washes, and contamination from modern rootlets was minimised by passing the sample through a 250-μm mesh sieve. Dates were obtained on the resultant fine particulate fraction (F) as Dresser[3] had previously found this gave the most reliable estimate of the true radiocarbon age of blanket peat samples.

The pretreated samples were burnt, and after purification, the resultant carbon dioxide was mixed with hydrogen, passed over a ruthenium catalyst and converted into methane. The ^{14}C activity of the methane was measured at a pressure of 2.5 bar in a conventional 2-l copper gas proportional counter which was surrounded by a multi-anode anticoincidence counter and 8 ton of pre-nuclear age steel. Quoted precisions of the dates are one standard deviation and derive mainly from counting statistics.

The accumulation rate of blanket peats is variable[4]: at depth, the rate may be of the order of 50–100 yr cm^{-1}, but towards the surface where autocompaction is not as intense and the peat less humified, the accumulation rate may seem greater. Microscopic examination of the Cefn Ffordd profile revealed particles of coal dust in the upper 2 cm thought to correspond with modern coalmining. From these two lines of evidence a rough estimate of 100–250 yr BP was made for the inferred heather moorland–grassland transition. However, the radiocarbon date for a sample at 4–5 cm was 2,970 ± 50 yr BP (CAR-47), some 2,000 yr older than a sample from 8 to 10 cm which was dated to 925 ± 45 yr BP (CAR-106).

Samples from the Coed Taf site were dated subsequently using the same pretreatment. In the upper 2 cm, particles akin to soot were detected but no coal dust was observed. A date of 140 ± 50 yr BP (CAR-81; 1,810 ± 50 yr AD (ref. 5)) was obtained for a sample at 11–13 cm, which strongly suggested that the peat of a sample at 6–8 cm would have accumulated after the start of the industrial revolution in Wales. The radiocarbon age of this sample was 3,710 ± 90 yr BP (CAR-82F).

Thus at two sites, 11-km apart, upper horizons of peat produced radiocarbon dates that were apparently too old. Both samples pre-date similar changes in pollen spectra from which one might infer a similar vegetational change.

These old dates needed to be explained. Both were checked. Laboratory contamination could almost certainly be ruled out as contamination during pretreatment would be more likely to introduce modern (more radioactive) than older carbon.

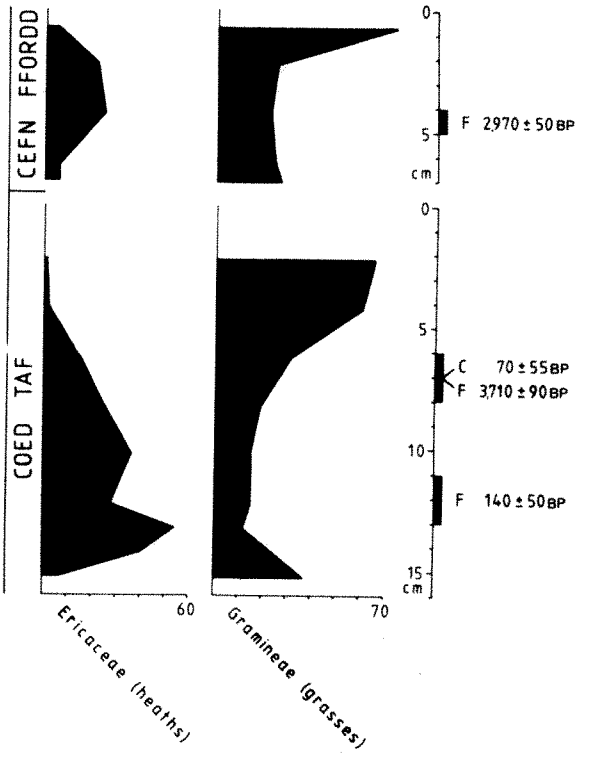


Fig. 2 Ericaceae and Gramineae curves (percentage total land pollen) for surface horizons of Cefn Ffordd and Coed Taf. Radiocarbon dates on humic acid (C) and fine particulate (F) fractions shown. For laboratory code numbers, see text.

Contamination during peat accumulation by material derived from older layers must, however, be considered. Eroding peat can be redeposited by water or wind. Either explanation is improbable because: (1) gross contamination from older peats would have been required to produce dates so old. Redeposition on this scale can be fairly easily recognised in the field but was not observed at either site. (2) Even the basal peat at Coed Taf is markedly younger than the date obtained for the fine particulate material at 6–8 cm. (3) Both sites were deliberately selected as being on convexities where inwash would be minimal. (4) There seem to be no recently eroded areas nearby that could have produced either windblown or waterborne organic material.

The incorporation of ancient carbon into a sample would dilute the activity of contemporary carbon, but there was no visual evidence of coal dust within the horizons dated. Although soot was used as a fertiliser during the nineteenth century, this seems to have been restricted to areas of market gardening or for improving heavy clay soils; its use on moorland in South Wales is not mentioned by Osborne[6]. Incompletely burnt fossil fuel (such as smoke and smut), carried up above the industrial valleys and deposited or rained out onto the moorland[7], seemed to be the only feasible source of old carbon.

This hypothesis was tested by dating the humic acid component of the 6–8-cm sample from Coed Taf. If the peat were as old as the F-fraction date had suggested, then the humic acid (C-fraction) would be expected to be of comparable age, or slightly younger[3,8]. The date obtained was considerably younger at 70 ± 55 yr BP (CAR-82C). This result could be obtained only from organic material produced after the start of the industrial revolution.

The implications of these results may now be considered.

(1) Atmospheric pollution produced by the substantially increased exploitation of coal during the industrial revolution has been incorporated in these South Wales upland peats. The discovery of this by radiocarbon dating enables some quantitative estimate of the scale of pollution to be made. Carbon from coal contains no detectable ^{14}C. In order for the fine particles of true age 100 yr BP to produce such old ^{14}C dates, more than 30% contamination of the organic carbon fraction by inactive carbon would be required. Assuming the apparent increase in age was due to the incorporation of inactive carbon, this would seem to comprise $36.4 \pm 0.8\%$ of the carbon content of the fine fraction of the Coed Taf 6–8-cm sample. Based on data from the combustion of the F-fraction in the dating process, and assuming that all the inert carbon was contained in this component, the original (untreated) peat sample contained 0.83 ± 0.01 g inactive carbon. This sample had an area of ~ 64 cm^2 and was accumulated over ~ 32 yr (based on the peat accumulation rate). These figures indicate there was an inactive carbon deposition rate of the order of 4 g yr^{-1} m^{-2}. (The original work did not envisage this type of treatment, but a subjective appraisal of the errors involved indicates an error of ± 2 g yr^{-1} m^{-2}.). The Cefn Ffordd inactive carbon deposition rate was of the order of 2.5 g yr^{-1} m^{-2}.

(2) In the radiocarbon dating of recent moorland vegetational changes near industrial areas, the use of unpretreated peat, or certain components, is likely to produce an erroneous result. Further research is still required, however, to ascertain the reliability of dates on the humic acid fraction as this component of blanket peats has already been shown to yield dates which are slightly too young[8].

(3) The transition from heather moorland to *Molinia* moor seems to have taken place at the two sites after the start of the industrial revolution. Did, then, changing moorland management practices associated with disrupted traditional employment patterns bring about such a marked vegetational change, or was the recent degeneration of heather moorland in some way connected with airborne pollution? Conway was inclined to discount the influence of atmospheric pollution on Pennine bogs[9], but the scale may not have been fully appreciated: deposition of some 0.6–1.8 ton per hectare inactive carbon over a 30-yr time-span is indicated for these South Wales moorlands. These figures convert to between 5.5 and 16.5 mg m^{-2} d^{-1} when expressed in the form used for modern deposit-gauge observations on undissolved deposits and ash yields[10].

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Agnathans from the Devonian of China

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Our Anglo-Chinese re-examination of material from southern China has led to a reappraisal of the two major groups of the primitive armoured jawless fish, the ostracoderms. We have united the galeaspids and polybranchiaspids in the Galeaspida of equal rank to the Osteostraci (Cephalaspides) and grouped them within the same major agnathan division as the anaspids and lampreys—the Cephalaspidomorphi. We also now believe that during the Lower Devonian, southern China was part of an isolated faunal province in which the agnathans experienced a major evolutionary radiation, independently from the rest of the world.

Fossil agnathans were first listed from southern China in 1937 by Ting and Wang[1] and, during the International Geological Congress in 1948, Young[2] reported rich deposits of Devonian vertebrates containing the agnathan *Cephalaspis*. This material was never described and its whereabouts are unknown. However, work in the same area in the same horizons in the early 1960s yielded several unusual forms[3]. The new genera *Galeaspis* (Fig. 1) and *Nanpanaspis* (Fig. 3*b*) were assigned to the Osteostraci (Cephalaspides), while *Polybranchiaspis* (Fig. 2) was placed in the Heterostraci (Pteraspides).

These fossils were unlike any previously described agnathans and their relationships to the known major groupings became a subject of controversy. It was suggested that they represented a new group of Agnatha to which the name Galeaspida was given[4,5]. This view received support from Stensiö[6] and Janvier[7] but was rejected by Miles[8]. Novitskaya[9,10] considered that the polybranchiaspids possessed a combination of osteostracan and heterostracan features and hence concluded that there was "no complete morphological break" between these two groups.

In the 1970s many new agnathan genera were described[11–14], including *Laxaspis*, *Sanqiaspis*, *Huanaspis*, *Asiaspis* and *Lungmenshanaspis* (Fig. 3), all of which were classified with the heterostracans. P'an[13] erected a new order of Heterostraci, on the basis of fragmentary remains of the genus *Hanyangaspis*. (This genus has been restudied and two of us (L.B.H. and Y.-H.L.) consider that it can be interpreted as a polybranchiaspid[15].)

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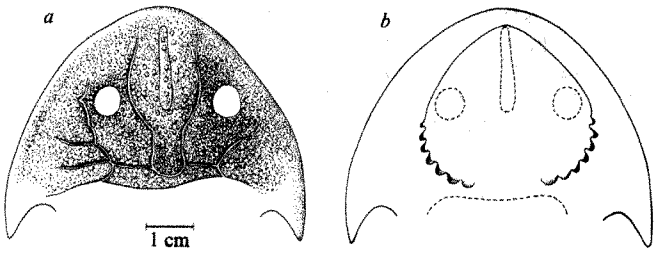


Fig. 1 Restoration of *Galeaspis* based on holotypes of *G. changi* Liu and *G. xujiachongensis* Liu. *a*, Dorsal view showing median dorsal opening, orbits, posterolateral spines and sensory canal system. *b*, Ventral view showing branchial openings and outline of buccal floor (IVPP, Peking).

P'an and Wang[14] identified the new genus (*Nakaolinaspis*) as a member of the amphiaspid heterostracans, a group confined to the Devonian of Siberia[16]. A restudy of this genus has convinced us that it was based on part of the skull roof of a specialised placoderm and hence there is no evidence that the amphiaspids are represented among the Chinese agnathans.

Southern China was clearly a province where agnathans underwent a major radiation. They were different from the familiar groups known from other parts of the world and their structure and relationships were controversial. We have re-examined the material in the Geology Museum and Institute of Vertebrate Palaeontology and Palaeoanthropology in Peking and reappraised the relationships.

The galeaspids (s.s.) and polybranchiaspids both possess a bony carapace covering the anterior part of the body, both dorsally and laterally. The lateral margin forms a longitudinal carina which extends on to the ventral surface and may be drawn out to form posteriorly or laterally directed spines. Similarly the anterior margin of the carapace forms an anterior ventral rim which is continuous with the lateral margin and may also be drawn out into an anterior projection or rostral spine.

The dorsal surface of the carapace is perforated by three openings—towards the anterior margin, a pair of circular openings which may be dorsally or laterally placed, and a median opening which may be transversely oval (*Polybranchiaspis*), circular (*Duyunaspis*), arcuate and transversely elongated (*Sanqiaspis*) or narrow and longitudinal (*Galeaspis*). On the inner surface of the armour there is a small round depression behind the dorsal median opening, which does not penetrate the carapace.

The inner margin of the ventral rim forms a smooth arc which runs posteriorly to the level of the paired circular perforations, behind which level the ventral rim at its median border forms a series of smooth notches. In some specimens small oval or circular plates are preserved adjacent to these notches and partially covering them.

The central median dorsal opening has a rim of fine ornament on its inner margin and in well preserved specimens the opening is raised somewhat above the general level of the rest of the carapace. In many specimens there is a well demarcated moat-like groove in front of the anterior margin of the opening. In a few specimens of *Polybranchiaspis* it can be seen that this opening was covered in life by an oval plate (Fig. 4). (Studies of photographs by J. A. Halstead and L.B.H. have revealed the presence of a similar plate in *Galeaspis*.) Behind the carapace in some specimens the squamation is preserved. On the ventral surface of the tail there are small diamond-shaped scales, the lateral scales are narrow and deep; in some forms there are dorsal ridge scales. There is no evidence of movable paired appendages and in *Sanqiaspis* it can be demonstrated that the tail was reversed heterocercal or hypocercal.

The sensory canal system comprises a similar pattern, as can be seen in Figs 1 and 2. The preservation is also comparable in both groups, in that the canals are enclosed within the armour. Frequently, as in *Polybranchiaspis*, they appear to lie in grooves on the surface, but in well preserved specimens they are covered by the superficial ornament (Figs 2 and 4*b*).

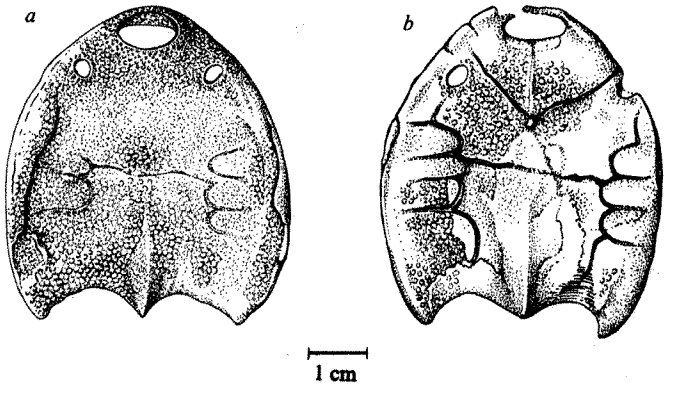


Fig. 2 *Polybranchiaspis liaojiaoshensis* Liu. *a*, Dorsal view showing median dorsal opening and dorsal orbits, with superficial ornamentation covering sensory canal system. *b*, Holotype, in dorsal view with outer surface removed to expose underlying sensory canal system (IVPP, Peking).

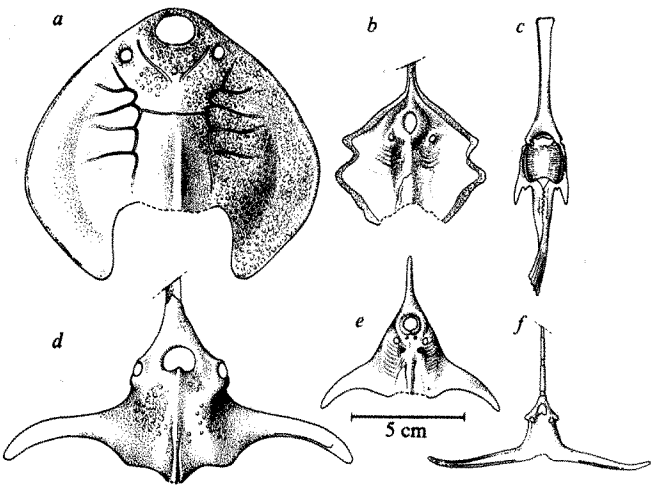


Fig. 3 Galeaspid genera in dorsal view to illustrate variety of external form. *a*, *Laxaspis* Liu; *b*, *Nanpanaspis* Liu; *c*, *Sanqiaspis* Liu; *d*, *Huanaspis* Liu (IVPP Peking); *e*, *Asiaspis* P'an; *f*, *Lungmenshanaspis* P'an and Wang (Geological Museum, Peking).

Many of the morphological features dealt with here have never been the subject of disagreement. It is universally accepted that the paired circular dorsal or lateral perforations of the carapace must be the orbits, while the small rounded depression, behind the dorsal median opening, is the position of the pineal organ. Similarly the notches on the median margin of the ventral part of the lateral extension of the dorsal carapace represent the individual openings of the gills or gill pouches.

The most controversial structure is the dorsal median opening, previously interpreted as a nasohypophyseal opening in *Galeaspis* and a mouth in *Polybranchiaspis*. The size of the opening seems to militate against it being nasohypophyseal, yet the position and profile seem inappropriate for a mouth. The discovery of a cover plate *in situ* in *Polybranchiaspis* and *Galeaspis* (Fig. 4) shows that it could not have functioned as a mouth and hence the inner margin of the ventral rim must have marked the anterior margin of the mouth, as in other agnathans such as cephalaspids and heterostracans. We suggest that the dorsal opening housed a sensory organ concerned with olfaction. There is no evidence as to whether this structure served as an inlet for water into the buccal cavity and hence to the gills, as a kind of analogue of the spiracle found in amphiaspid heterostracans and living skates and rays. Certainly for a benthonic

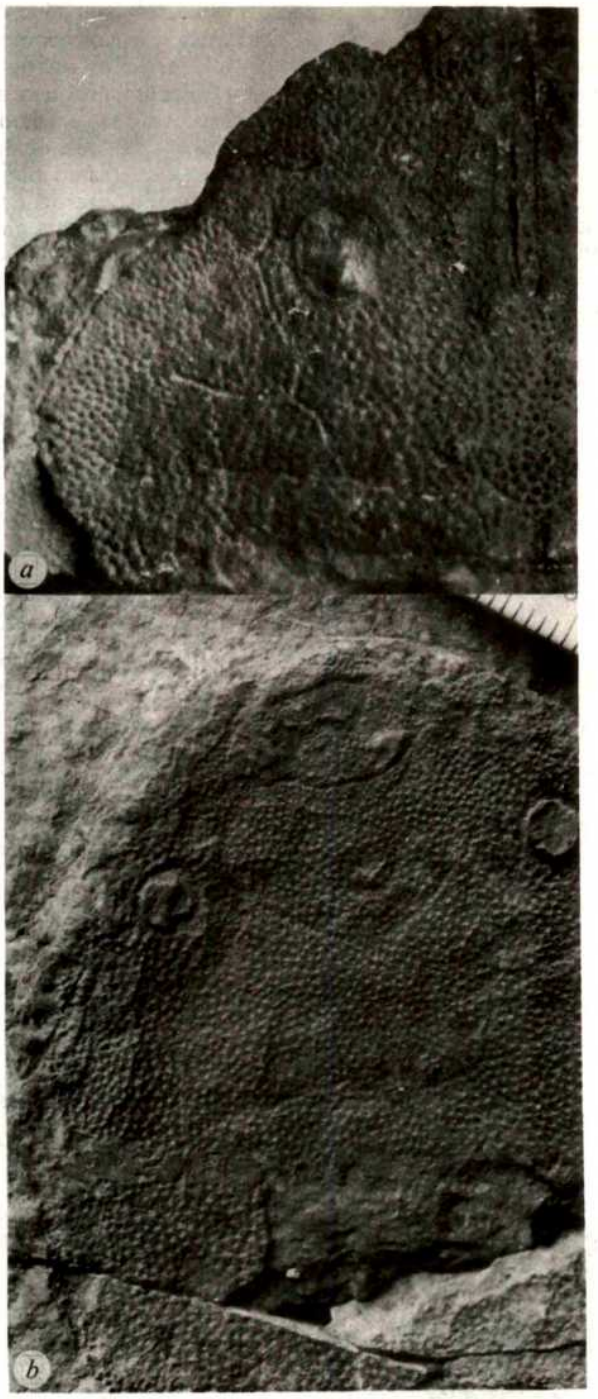


Fig. 4 *a*, *Galeaspis changi* Liu, holotype, dorsal view showing cover plate in position over median dorsal opening (IVPP, Peking). *b*, *Polybranchiaspis liaojiaoshensis* Liu, dorsal view showing cover plate in position over central median dorsal opening (IVPP, Peking).

microphagous feeder, it would make good functional sense for this opening to have served such a purpose.

From our examination of the morphology of the galeaspids (s.s.) and polybranchiaspids and the interpretations of the structures to which we have now been led, we agree that both groups can be united into a single major taxon of high rank, the Galeaspida, equivalent to Osteostraci and Anaspida.

The Galeaspida, Osteostraci and Anaspida all have three main dorsal openings, and separate gill openings. The first two groups have similarly preserved endocranial connective tissue[12,17]. Features which appear to link the Galeaspida with the Heterostraci include possible double nasal organs, a simple brain, a pineal body covered by the external armour and the overall shape of the carapace and hypocercal tail. These characters cannot be considered diagnostic as they are all primitive and in some cases would apply to such primitive cephalaspids as the trematapsids. The Chinese agnathans can be excluded from association with the heterostracans but they can be linked in a general way with the cephalaspids.

With the overall systematic position of the galeaspids agreed in the context of the known agnathan groups, it is evident that *Galeaspis* closely resembles the cephalaspids in having its gill openings arranged into a more or less circular pattern and also in having fewer gill openings than do the polybranchiaspids. The latter seem to be less specialised in this regard, with gills arranged serially along the anterior part of the body, although in the overall proportions of the carapace the polybranchiaspids vary greatly. The apparent similarity of *Galeaspis* to the cephalaspids, with the exception of the presence of lateral and posterior dorsal sensory fields, may indicate a degree of direct relationship or parallel evolution as the organisms become adapted for an ecological niche comparable to that occupied elsewhere by the cephalaspids.

The Galeaspida represent a unique major evolutionary radiation of the ostracoderms, which appears on present evidence to have been confined to the south China province. With the possible exception of the Silurian cyathaspid *Kiangsuaspis* described by P'an[18] there is no sign of any agnathan that can be linked to forms known from other parts of the world. The firm impression is that, as far as agnathans were concerned, this region was isolated during the Lower Devonian.

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Internal anatomy of the polybranchiaspids (Agnatha, Galeaspida)

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A number of polybranchiaspids (Agnatha) from the Devonian of China have parts of their soft anatomy, including the central nervous and vascular systems, extremely well preserved as mineral replicas. This has made possible direct comparisons with the internal anatomy of the living agnathans, the hagfish and lampreys, as well as the fossil heterostracans and cephalaspids. The polybranchiaspids do not seem to be related to either the hagfish or the heterostracans, but instead represent a separate grouping, the Galeaspida, allied to the lampreys and cephalaspids.

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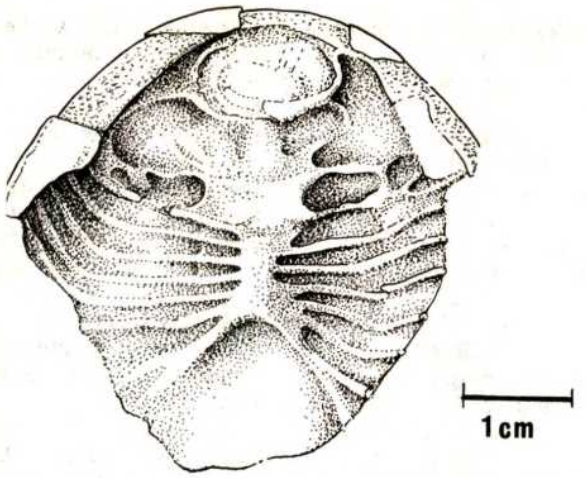


Fig. 1 *Polybranchiaspis liaojiaoshensis* Liu, V.3027, Institute of Vertebrate Palaeontology and Palaeoanthropology, Peking, internal view of perichondral ossification of endocranial tissue showing mouldings produced by orbital sinus and somites.

The anatomy of the polybranchiaspid carapace is now well known[1–4]. The neurocranial floor on the inner surface of the dorsal carapace of *Polybranchiaspis* (Fig. 1) was described by Liu[2]. Beneath the superficial ornamentation at the same level as the sensory canal system, there was a subaponeurotic (subcutaneous) vascular plexus (Fig. 2).

P'an and Wang[3] described several polybranchiaspid specimens, in particular *Duyunaspis*, in which after death the spaces occupied by the central nervous and vascular systems were filled by a secondary iron mineral and the bony material had dissolved away to leave a mineral replica of the internal anatomy (Fig. 3).

There are only two pairs of semi-circular canals in the ear, the anterior and posterior vertical, both with clearly defined ampullae. The two canals meet medially as the point where the utriculus is situated and from where it develops ventrolaterally. The sacculus, in contrast, is frequently expanded dorsomedially so that it comes to lie medial to the semi-circular canals. The round structure arising from beneath the canals and situated between them and the brain, is best interpreted as the sacculus.

The position of the orbits is in the region labelled 'premandibular elevation' by P'an and Wang[3]. The eyes are unlikely to be adjacent to the dorsal median opening but rather as in other polybranchiaspid genera[1–4]. The position of the nasal sacs is more difficult to decide. There is no justification for the positioning of the nasal sacs at the anterior margin of the specimen[3], a more likely position is immediately lateral to the dorsal median opening. There appears to be a nerve running from the forebrain region to this structure and, on this interpretation, this would be the olfactory nerve. The other possibility is the two structures immediately behind the dorsal median opening which P'an and Wang[3] identified as 'possible nasal lobes'.

The dorsal median opening is transversely oval but a posteriorly directed V at its posterior margin indicates a canal leading to the hypophysis, suggesting that the dorsal median cavity was a nasohypophyseal structure. There is no evidence as to whether this opened directly into the buccal cavity as in hagfish or was blind as in lampreys and cephalaspids.

The infilling of the endocranial cavity indicates the general configuration of the brain. The spinal cord with its accompanying spinal nerves expands anteriorly into the medulla oblongata, the major part of the hindbrain. There is no evidence of a transverse commissure representing the cerebellum or additional lobes relating to the development of the acousticolateralis system. The constriction lying at the junction of the anterior and posterior semi-circular canals of the ear was the position of the midbrain, even though in higher vertebrates it is always situated anterior to the ear region. In the polybranchiaspids, the pineal organ was sited at the level of the anterior semi-circular canals and therefore the midbrain must have been posterior to it.

The anterior part of the forebrain, the telencephalon, is either represented by the pair of irregular structures laterally or by the region lateral and deep to the pineal. In either interpretation, a degree of cranial flexure occurred as a consequence of the development of the median dorsal organ.

The identification of the cranial nerves that can be observed, presents considererable difficulties and hence must be considered tentative. The two cranial nerves seen on the right running obliquely posterolaterally were probably the different branches of the vagus X, and the nerve anterior to these but posterior to the ear region, the glossopharyngeal IX. Two nerves in close proximity running anterolaterally but diverging somewhat and approaching the brain beneath the semi-circular canals of the ear were the facial VII and trigeminal V nerves.

The most prominent feature of the vascular system was the pair of large calibre vessels running on either side of the labyrinth of the ear and the brain, the *vena capitis lateralis*[3] or lateral head vein, anteriorly ending in a large orbital sinus. Posteriorly there was the occipital venous sinus. It is not possible to determine with any certainty at which point the veins from the anterior and posterior parts of the body joined to form the common cardinal vein or *ductus Cuvieri* leading to the heart, and for this reason topographical terms are used. A series of segmental veins leading into the *vena capitis lateralis* are clearly preserved. Lateral to the occipital venous sinuses were further vessels draining segmental structures, paired dorsal aortae, from the efferent arterial system.

The most prominent aspect of the internal anatomy of the polybranchiaspids, observed in most genera[3,4] were a series of paired (up to 20 in number) dorsally positioned structures rising up on either side of the central nervous system, extending laterally some distance, and then curving posterolaterally and each associated with a separate branchial opening along the ventrolateral margin of the carapace. Such dorsal structures have been interpreted as representing the former position of gill



Fig. 2 *Polybranchiaspis liaojiaoshensis* Liu, portion of subaponeurotic (subcutaneous) vascular plexus and sensory canal system enclosed within the dermal armour.

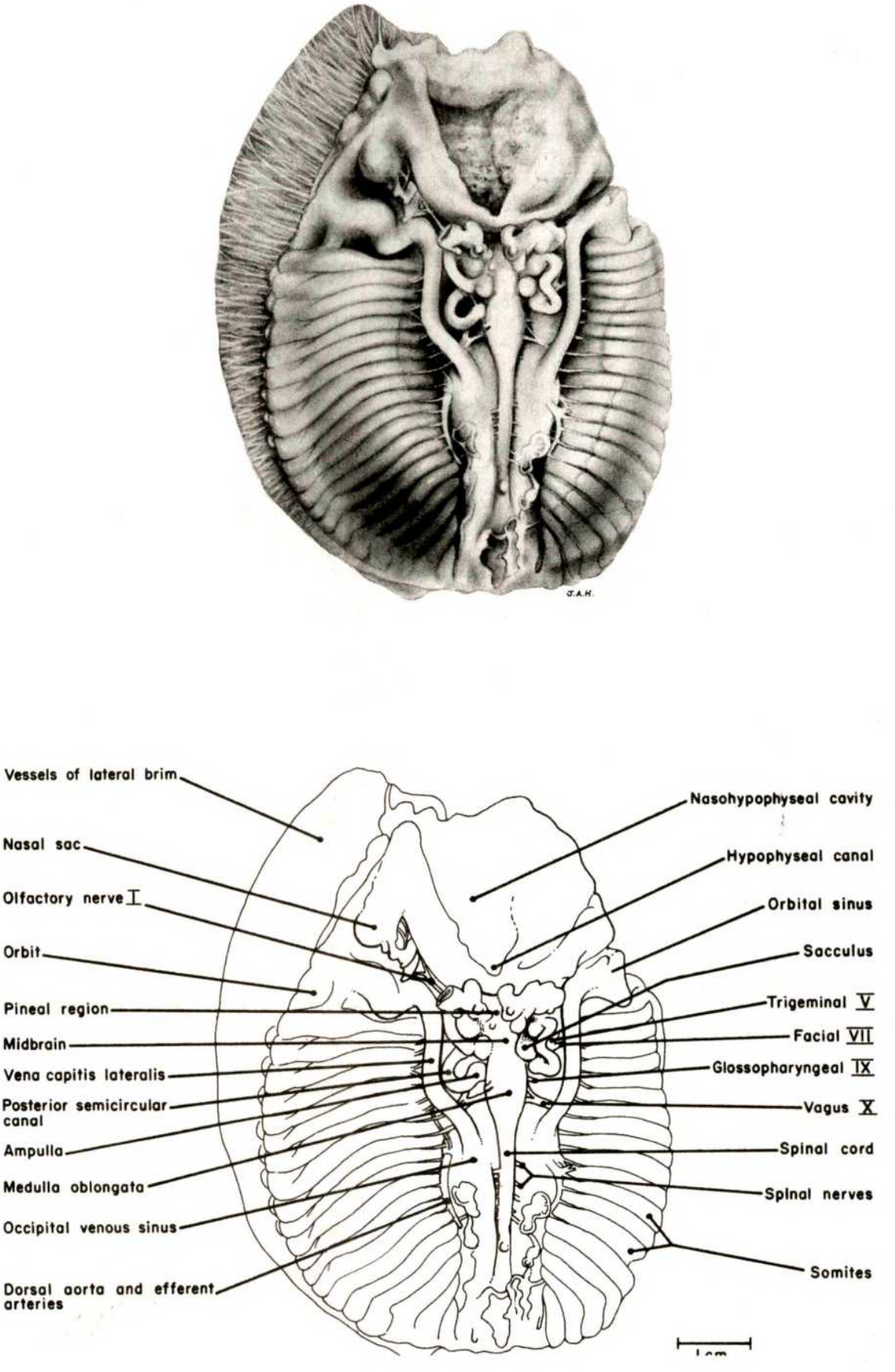


Fig. 3 *Duyunaspis paoyangensis* P'an and Wang, holotype, V.1324, Museum of Geology, Peking, in dorsal view showing mineral infilling of canals and spaces in endocranial connective tissue which have become exposed subsequent to the removal of the original calcified tissues. Identification of structures indicated in accompanying annotated diagram.

pouches[3,5], but also as somites or segmental muscle blocks[6,7]. The polybranchiaspids did not acquire their bony armour until they achieved their definitive size, hence it is necessary to postulate the existence, in the young stages, of serially arranged somites on either side of the central nervous system along the dorsal part of the head and trunk region.

These structures can be interpreted as segmental muscle blocks above the gill pouches, contributing to the pumping mechanism. The gills were unlikely to have been in direct contact with the dorsal armour; there would have been some somitic musculature intervening.

The preservation of the internal anatomy of the polybranchiaspids makes possible direct comparisons with living agnathans, the hagfish[8] and lampreys[9,10], as well as fossil, cephalaspids and heterostracans[6,7,11-14]. The living forms are characterised by their eel-like shape and absence of a bony external skeleton, scales or paired fins, all of which appear to have been secondarily lost. Several further features unite the two groups: a single external nasohypophyseal opening, paired olfactory lobes, pore-like gill openings, horny teeth on the tongue and the haemoglobin molecule made up of a single polypeptide chain (in contrast to the four of all higher vertebrates). Many of the differences are connected with their different modes of life. The hagfish is highly specialised in its reduction of sense organs, the eyes are reduced to the merest vestiges, there is no pineal organ and only a single canal in the ear, generally considered homologous to the posterior semicircular canal and a combined utriculus-sacculus element. The only feature that can be directly compared with that of the polybranchiaspids is the nasohypophyseal opening, which is terminal. The venous system of the hagfish, with its ventral cardinal 'heart' deep to the anterior cardinal sinus, is not comparable to the lateral head vein and occipital sinus of polybranchiaspids. The well developed eyes and ear regions and the configuration of the brain of the polybranchiaspids render hagfish affinities unlikely.

When the polybranchiaspids are compared with the lamprey, the similarities are striking: there is a dorsal median opening with a pineal organ behind. There are two semi-circular canals of the ear and the configuration of the brain is similar. The gill pouches each had separate openings to the exterior as in lampreys. In contrast, however, the polybranchiaspids appear to have paired nasal sacs and the pineal was covered by the bony armour. The details of the internal anatomy strongly indicate a close relationship to lampreys. The cephalaspids possessed the same basic similarities and differences with the polybranchiaspids as the lampreys, but show further similarities in the degree of endocranial ossification and in the venous system, which is comparable to that of the primitive cephalaspid *Trematapsis*. The polybranchiaspids were closely related to the cephalaspids, but can be excluded from them on account of their lack of dorsal and lateral sensory fields, the corresponding lobes of the hindbrain, the large dorsal median opening, covered pineal and paired nasal capsules.

Finally, the polybranchiaspids can be compared with the heterostracans, with which they have been generally associated. They are similar in possessing double nasal sacs and a covered pineal but differ in the endocranial ossification and separate gill openings. The recognition that the dorsal median opening was not a mouth, excludes the polybranchiaspids from the heterostracans[4].

The details of the internal anatomy confirm that the polybranchiaspids belong to an independent group, the Galeaspida, allied to lampreys and cephalaspids within the Cephalaspidomorphi division of the Agnatha.

Specimens were drawn in Peking by Jennifer Halstead.

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Dioecious *Solanum* species of hermaphroditic origin is an example of a broad convergence

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Unlike the taxa in some genera which show a range of variability in floral morphology, species of *Solanum* deviate very little from the typical hermaphroditic flower. The known deviant taxa fall into two classes: most are andromonoecious (male and hermaphroditic flowers on the same plant), and the remaining few are androdioecious (male flowers on one plant, hermaphroditic flowers on another)[1]. I report here evidence for the first instance of a truly dioecious species of *Solanum*, a significant discovery in the light of the floral uniformity of this large (second only to *Senecio*) and economically important genus. The most closely related species and species groups comprise only hermaphroditic forms; this dioecious species is among the few non-hermaphroditic forms native to the New World, the area of greatest species diversity of the genus. Furthermore, as shown below, the species is characterised by a functionally, though not morphologically, dioecious condition. Finally, this demonstration that the two floral forms represent different sexes of the same species, rather than (as originally described) different species, impels closer analysis of other species pairs.

The plants studied were originally collected in mountainous regions of the states of Mexico and Michoacan in Mexico, and San Marcos in Guatemala. The specimens collected were identified using Correll's monograph[2] as *Solanum appendiculatum* and *Solanum inscendens*. These species are non-tuberous (although relatively closely related to the potato) woody perennials that grow to a length of 3-10 m up tree trunks and rock surfaces. The primary character used to separate these two species in keys is the length of the style in relation to the staminal column (short style, *S. appendiculatum*; long style, *S. inscendens*). When the initial collections were grown in greenhouses, seeds from each fruit collected produced both long style and short style plants. Subsequently, seed populations from 24 collections have been grown to generate the sex ratio given in Table 1 line (1). It is clear from ratios derived from populations of seeds collected in the field, and from a small artificially derived F_1 population (line 2), that the primary sex ratio is about two long (L-functional female, see below) to one short (S-functional male). However, the sex ratio derived from randomly selected populations in the field is nearer to 1 : 1. The latter ratio is closer to that usually reported for dioecious taxa[3-5]. At present, too little is known of the field biology of this *Solanum* taxon to speculate on which factors alter the primary sex ratio.

The relationship between the long- and short-style plants was assayed with crossing studies and with observations of pollen tube growth in styles. The results of these two analyses provide strong evidence that this species is functionally dioecious. More than 400 crosses were attempted among the four possible combinations of short- and long-styled flowers. The only successful combination involved L♀ and S♂ (Table 2). Seeds resulting from this combination were viable, and the mature F_1 offspring were fully fertile. Studies of pollen tube growth[6] add

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Table 1 Features of the sex forms

Features	Long-style form L	Short-style form S
(1) Sex ratio (greenhouse)	2.02 (107)	1.0 (53)
(2) Sex ratio in a greenhouse F_1 population	1.75 (7)	1.0 (4)
(3) Sex ratio (field collections)	1.0 (23)	1.22 (28)
(4) Flowers per inflorescence	34.0 (30)	34.27 (30)
(5) Pollen grains per flower	82,750 (6)	129,465 (5)
(6) Pollen stainability	78.6% (119)	79.4% (77)
(7) % Pollen grains: inaperturate	100%	0%
tricolporate	0% (2,281 grains, 20 collections)	100% (2,587 grains, 25 collections)
(8) Ovules per ovary	11.26 (42)	11.28 (36)

The upper of the two sets of numbers represent averages, while the lower sets, in parentheses, represent sample sizes.

further confirmation that the L♀×S♂ combination is the only one functional. With a single exception, pollen tubes were produced only when pollen from short-style flowers was placed on stigmas of long-style flowers (Table 2). Virtually all other combinations, including attempts at self-pollination (columns *E* and *F*, Table 2), failed to yield pollen tubes. These data are also interesting in relation to the question of potential mechanisms responsible for crossing success. Perhaps crosses fail and no pollen tubes are produced because of chemical interactions between stigma and pollen, such as those involved in typical S-allele (incompatibility) systems[7]. Or perhaps, as implied below, the pollen from the long-style flowers is physically unable to germinate.

Analysis of field collections and greenhouse-grown plants indicates that both long- and short-style forms possess an equal number of flowers per inflorescence (Table 1, line 4). This 1:1 ratio for inflorescence size is incongruent with the usually larger inflorescences of males reported by Opler and Bawa[4] for dioecious tropical trees. There is, of course, no reason to expect that every species will follow the general pattern Opler and Bawa have described. Furthermore, because *Solanum* flowers reward potential pollinators with pollen rather than nectar[1], one could expect the female inflorescences to remain as large as the male and thus not suffer a reduced frequency of visits because of insufficient reward.

Although the sex ratio is not male biased and inflorescence sizes are the same, counts[8] of the number of pollen grains per flower (Table 1, line 5) in the two sex forms are significantly different. The short-style flowers bear greater than 55% more pollen grains. Perhaps the smaller quantity of pollen grains in the female flowers (though not so small as to reduce the frequency of visits by pollinators) is one manifestation of the selection for unisexuality. Although the quantity of pollen is less in the long-styled flowers, two tests of viability (aniline blue in lactophenol, acetocarmine) showed that both flower types produced equally stainable pollen (Table 1, line 6). However, such tests can only estimate viability and not whether pollen is actually functional. The data given here for crossing and pollen tube growth are one indication that the pollen from long-style flowers is not functional. Light and scanning electron microscope studies of acetolysed pollen give another. The long-style flowers always bear inaperturate grains and the short-style flowers the more typical tricolporate types[9]. The lack of development of colpae and pores in the pollen of the long-style form may be one of a suite of adaptations to functional femaleness. Inaperturate pollen, presumably quite functional, is reported from several families including the Solanaceae[10]. However, the data reported here seem to be a strong indication that while pollen from the long-style flowers may be a suitable reward for floral visitors, it is not otherwise involved in the reproductive process.

Except for the difference in style lengths, the gynoecium (stigma, style and ovary) shows few morphological modifications. Contrary to expectations, the short-style flowers bear as many ovules as the long-style flowers (Table 1, line 8). This seems a strong indication that the dioecy in this species is still in an early stage of evolution.

The discovery of a new breeding system in a genus as large and invariable for this feature as *Solanum* is unusual. The important point is that the long- and short-style flower forms do not represent two species which hybridise. Crosses in the right combinations can be made with ease, and the resultant F_1 plants are fully fertile. In addition, greenhouse populations consistently give a 2L(♀):1S(♂) segregation ratio. Furthermore, the data for crossing and pollen tube growth show clearly that this is not a case of heterostyly. This is in agreement with previous conclusions that true heterostyly is unknown in the Solanaceae[11]. Rather, the long- and short-style flowers are simply breeding morphs of a single dioecious taxon.

What evolutionary advantage the dioecious system confers on this taxon is perplexing. The usual explanation that dioecy arises in a self-compatible taxon as a way of ensuring outcrossing[3,12] is certainly possible. However, it is somewhat less likely here because most closely related species tested possess a well developed system enforcing obligate outcrossing[7,13]. Bawa and Opler[3] have argued that dioecy may be selected in hermaphroditic flowers in preference to outbreeding mechanisms such as S-alleles to enhance effective pollination. They argue that if the number of flowers on an individual is high, most pollination will involve transfer of ineffective self pollen. Such pollen could cover the stigmatic surface so that pollen brought from another plant may, although capable of it, be unable to effect pollination because stigmatic space is unavailable. This argument was proposed for tropical trees with a very large number of flowers. It may apply to the dioecious *Solanum* described here because it has larger inflorescences with more flowers open at any one time than most closely related species[2,13]. However, the fact that females bear anthers with pollen (although significantly less pollen) lessens the force of this argument for this species.

The dioecy described here is also of particular interest because there is no precedent for unusual sex forms in any of the closely related species groups[1]. Several authors[14,15] have argued that dioecious taxa frequently evolve from gynodioecious or less frequently androdioecious ancestors. Gynodioecious taxa are unknown in *Solanum*, and although androdioecious taxa have been reported, they are, like most species with unusual sex forms in the genus, native to the Old World and are placed in a different subgenus[1]. Thus the development of dioecy described here does not represent the culmination of a linear evolutionary

Table 2 Results from cross pollination and pollen tube studies

	Combinations *A* L♀×S♂	*B* S♀×L♂	*C* L♀×L♂*	*D* S♀×S♂*	*E* S self†	*F* L self†
Crosses attempted	102	101	102	102	0	0
Crosses successful	64	0	0	0		
% successful	63%	0%	0%	0%		
Styles: with pollen tubes	44	0	0	1	0	0
without pollen tubes	0	23	20	22	5	6

* Crosses between flowers of different plants with same style lengths.
† Crosses between flowers on the same plant.

series, but rather a broad convergence on a set of apparently advantageous breeding systems. Furthermore, Crowe[16] has suggested that most dioecious species evolved from monoecious (primarily) or heterostylous taxa; the lack of either of these conditions in *Solanum* precludes their application to this case. The fact that the separation of sexes is still only functional, and not morphological, very likely indicates a system in the early stages of evolutionary development. This situation provides a special opportunity which, as Crowe proposed[16], may lead to a better understanding of the origin and evolution of incompatibility mechanisms among the angiosperms in general.

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Length and evolutionary stability of food chains

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Grazing food chains in natural ecosystems tend to be relatively short, usually involving no more than four or five trophic levels[1–3]. Cohen[4] describes 19 food webs with a total of 102 top predators. Recent independent analyses of these data by Cohen[4] and Pimm (see ref. 5) show that most top predators occupy the third trophic level. However, as Slobodkin[3] has observed, "It is possible to conceive of predators that are small enough and ferocious enough to attempt to live off the top of a trophic pyramid, and thereby become a seventh, or even an eighth, trophic level. It is by no means clear why this situation has not occurred." Our answer, which we discuss here, incorporates energy transfer calculations introduced by Hutchinson[1] and Odum and Odum[2] within the framework of the evolutionarily stable strategy (ESS) developed by Maynard-Smith and Price[6,7]. We argue that food chains should collapse to the shortest possible length compatible with biochemical and physiological constraints. This minimal length is frequently three, and is largely independent of primary productivity.

Odum and Odum[2] consider the inefficiency of energy transfer. If each species can transmit only a fraction (usually between 5% and 20%) of its incoming energy to the next level, this sharply limits the length of food chains. Although any such limit scales as the logarithm of primary productivity, Pimm and Lawton[8] observe that 'food chains are not noticeably shorter in barren Arctic and Antarctic terrestrial ecosystems compared with a productive tropical savannah or the fish guilds of a tropical coral reef,' although primary productivity varies by a factor of 10^4. Hutchinson[2] proposes that pressure to increase predator efficiency could even shorten food chains. An efficient higher predator, with limited food supply, might eliminate his prey and be forced either to drop to a lower trophic level or to become extinct. Pimm and Lawton[8] propose a third explanation involving Lokta–Volterra dynamics (see refs 5, 9 for reviews). Our explanation considers the evolutionary stability of alternative trophic strategies. Trophic levels are defined by assigning plants the first trophic level, herbivores the second and primary carnivores the third. More generally, a species which feeds primarily on the nth trophic level is assigned the $(n+1)$st trophic level. The classical energy transfer argument states that lower trophic levels offer a richer thermodynamic environment (in terms of free energy) than do higher trophic levels. Following the analysis of optimal foraging and resource partitioning of MacArthur and Pianka[10], a predator's strategy may be described as optimal if the predator exploits the thermodynamically richest accessible environment. We shall argue that such optimal strategies are evolutionarily stable, whereas alternatives are not.

Consider a fourth-level species, one which feeds primarily on carnivores. Its nutritional needs could be satisfied at a lower trophic level by herbivores, as carnivores and herbivores are nutritionally interchangeable. In the absence of additional non-nutritive constraints, members of this species could always drop to a lower trophic level and compete with their former prey for the thermodynamically richer herbivore environment. As long as the marginal energy gain from feeding on herbivores is greater than feeding on carnivores, the ESS will be to concentrate on herbivores, but to feed on carnivores encountered in defence or competition. This assumption on energy gains is justified by the usual range of transfer efficiencies, and the probable greater unit costs of successfully attacking carnivores.

More precisely, let $h(t)$ be the energy gain from expending an effort t on feeding on herbivores. Similarly, let $c(t)$ be the gain from an effort t on carnivores. We assume that both $h(t)$ and $c(t)$

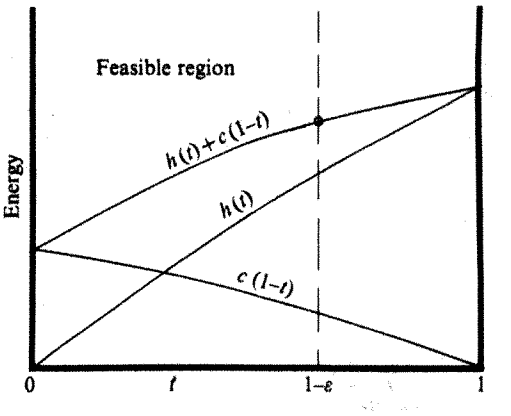


Fig. 1 The ESS of maximum feasible energy gain. The graph shows the net energy gain $h(t)+c(1-t)$ from a strategy of spending effort t on herbivores and $1-t$ on carnivores, its components $h(t)$ and $c(1-t)$ from herbivores and carnivores, and the required energy ε expended on feeding on carnivores. Thus, $0 \le t \le 1-\varepsilon$. The maximum energy gain occurs at $t=1-\varepsilon$ under our assumptions.

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are increasing functions, convex because of 'diminishing returns', and close to linear, and that $c(t) \ll h(t)$—the ratio $c(t)/h(t)$ represents an effective transfer efficiency. Now, consider a strategy of expending an effort t on feeding on herbivores and $1-t$ on feeding on carnivores. Here $0 \leq t \leq 1$. We seek to maximise the total energy gain $h(t)+c(1-t)$ subject to the constraint that the organism expend at least a small effort ε in competing with (and incidentally feeding on) potential carnivore prey. Many factors influence ε, including territorial behaviour, ability to flee and population density. As long as

$$\left.\frac{dh(\tau)}{d\tau}\right|_{\tau=t} > \left.\frac{dc(\tau)}{d\tau}\right|_{\tau=1-t}$$

or simply, $h'(t) > c'(1-t)$, for $0 \leq t \leq 1-\varepsilon$, this maximum will occur at $t = 1-\varepsilon$ (Fig. 1). Therefore, from the above assumptions, if relatively little energy ε need be expended for competition, such an organism should adopt the ESS of concentrating (spending effort $1-\varepsilon$) on herbivores.

The option of switching to a lower trophic level is not available to first-level carnivores because of nutritional differences between plants and herbivores; herbivores clearly face far stricter constraints. Furthermore, these nutritional requirements arise from synthetic and degradative specialisations which are selectively advantageous. However, a parasite may be subject to different dynamics, and not always select the most numerous host because its host constitutes its entire thermodynamic and spatial environment, and is not a potential competitor under alternative trophic strategies. Thus, on the spatial scale of an individual parasite[6,7], a herbivore host might offer no advantages over a carnivore. It is also conceivable, but energetically unlikely, that diminishing returns could make $h'(t_*) = c'(1-t_*)$ for some $t_* < 1-\varepsilon$. This yields a mixed strategy[6,7], with effort t_* expended on herbivores and $1-t_*$ on carnivores. Finally, the case of concave $h(t)$or $c(t)$ is also energetically unlikely.

We have shown that in the absence of non-nutritive constraints, grazing food chains should evolve towards the evolutionarily stable length of three. Parasitism and opportunistic feeding allow for some chain lengthening. Longer chains may also exist transiently, possibly being relatively long-lived, or may persist due to special constraints such as size and habitat compatibility of predator and prey. Differences in nutritional value of predators and prey are unlikely to be as significant for protista as for metazoans, but size constrains may be more important. These factors are independent of primary productivity. Further, our argument that optimal foraging subject to biochemical and physiological constraints is an ESS applies to all trophic levels and strategies, independently of the detailed structure of the food web. We therefore predict that the length of food chains will be relatively constant over wide ranges in primary productivity. This agrees well with field data[4,5].

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Male lions in large coalitions gain reproductive advantages

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Cooperation between two or more individuals has been shown to yield short-term benefits in several vertebrate species[1–4], and various hypotheses have been developed to explain the evolution of cooperative behaviour[5–7]. However, until now there has been no evidence to show that such cooperation actually does confer lifetime's reproductive advantages on more than one member of the coalitions concerned[8,9]. Long-term studies of wild lions (*Panthera leo* L.) have now provided such evidence. We show that, compared with singletons and pairs, male lions in groups of three or more can more reliably gain tenure of female prides, retain tenure for longer, mate with more different females, and produce more surviving offspring; thus each individual has higher fitness through cooperation.

Lions were observed in the Serengeti National Park and in the adjacent Ngorongoro Conservation Area, Tanzania, in 1966–69 by G. B. Schaller[2]; in 1969–73 by B.C.R.B.[10,11]; and in 1974–78 by J.D.B. and J.P.H.[12]. Observations on the same prides in comparable habitats were spread over periods of 3–12 years. All members of these prides were known individually by natural markings[13].

A lion pride is a matrilocal group of 2–18 (mean 6)[2,12,14] genetically related females, plus their cubs. Young male lions usually leave their natal pride as a group of relatives shortly before sexual maturity. Then, as a coalition, they gain and retain sole possession of a pride for a variable period, excluding rival male groups. The male group's tenure, and therefore its effective reproductive life[10], typically ends when they are displaced from their pride and their area by a new group of males. Here we compare aspects of the reproductive success of the male groups of different sizes.

Table 1 The frequency of male lion groups of different sizes, and the probability that they will have tenure of a pride of lionesses

No. of males in group	No. of male groups: Total	No. of male groups: No. which had tenure	Probability of having tenure	Significance of difference
1	23	4	17.4%	
2	39	23	59.0%	<0.01
3	12	11	91.7%	<0.01
4	4	4	100.0%	
5	1	1	100.0%	
6	3	3	100.0%	
7	1	1	100.0%	

Total 183 adult males (that is, ≥4 yrs old), observed in the Serengeti region over more than 2 yrs during 1969–78.

Table 1 shows that single males in the Serengeti region were rarely successful in gaining tenure of a pride (4/23), whereas pairs quite often were (23/39 pairs); large groups (3 or more males) were almost always in possession of a pride (20/21 groups).

Having gained tenure, large male groups were also more successful in retaining it. Data henceforth refer to 25 male

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groups. Figure 1*a* shows that compared with pairs (mean tenure 18 months), groups of 3 males stayed at least 2.3 times as long, and groups of six males stayed over 3.8 times as long with their prides. Note that the tenure periods of all the large groups (but of only some of the small groups) are underestimated because they extended beyond the beginning or end of the observation periods; thus the differences shown are conservative.

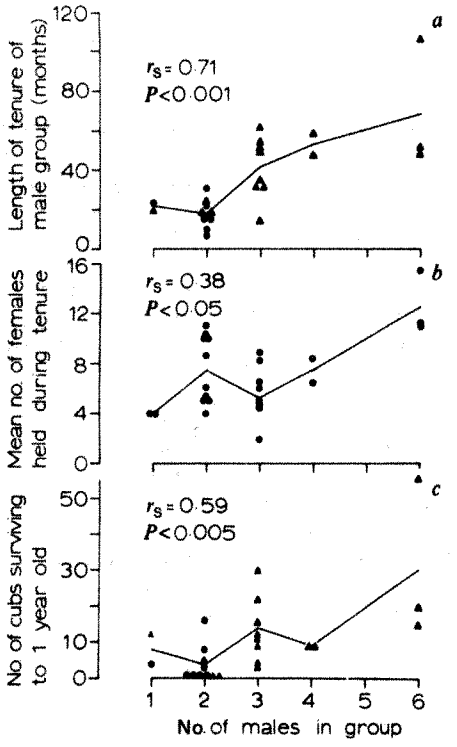


Fig. 1 Aspects of the reproductive success of male lion groups of different sizes. *a*, The numbers of months that each group retained tenure of a pride or prides in the study area. *b*, The mean numbers of females concurrently in the possession of the male groups, averaged over their observed period of tenure. *c*, The total numbers of cubs fathered during the male groups' tenure which survived to 1 yr old (by which age 77% of cub mortality has occurred[10]). ▲, Points which are minimum figures because the male groups' periods of tenure extended beyond the beginning or end of the study period; ●, points which are not underestimates.

Large male groups, especially after staying with one pride for about 2 years, may gain access to one or more neighbouring prides as well. Their concurrent tenure of several prides gives them access to more oestrous females. Figure 1*b* shows that in general only the very largest male groups (>4) held appreciably more females concurrently. They did so not by holding larger prides but by holding two or more at the same time.

When a lioness comes into oestrus (lasting 2–5 d), any male in the group consorts alone with her, mating repeatedly. Having companions should subdivide a male's share of consortships; however, the available data show no significant negative correlation between the number of males in a group and their consortship scores ($n = 50$, $r_s = +0.14$, $P > 0.2$. Consortship scores were calculated as a percentage of total sightings, excluding males sighted fewer than 10 times. For 50 males, $\bar{x} = 19.6\%$, s.d. = 8.6).

There is no detectable hierarchy within male groups[2,14], all partners sharing their mating duties with remarkable equity. For example, no male was seen consorting more than 2.7 times as often as his partner(s), and in the most often observed group of 6 males ($\bar{x} = 81$ sightings of each male) no male was involved in >22% or <9% of all consortships seen. Overall, the difference between consortship scores was smaller and far less variable between members of the same coalition ($\bar{x} = 7.6$, s.d. = 5.6, $n = 62$) than between members of different coalitions ($\bar{x} = 11.0$, s.d. = 26, $n = 1{,}138$; F test $P < 0.001$).

A female may sometimes change consorts during her oestrous period, and thus mate with more than one male. There are no indications that particular males are likely to be in consort with her at particular times during her oestrus. Copulation may occur as frequently as every 15 min, so only a minute proportion of copulations (<1%) leads to the birth of cubs[2,10]. Hence, unlike some species[15,16], for lions the number of copulations would not provide a reliable indicator even of short-term reproductive success. Nor would the number of litters born, because cub mortality is high and because high mortality of lion cubs raises the birth rate[10].

The best measure of lifetime reproductive success is the number of surviving offspring produced. Figure 1*c* shows that the total number of cubs surviving to 1 year old is correlated with their fathers' group size ($r_s = 0.59$, $P < 0.005$). The effect is achieved mainly by staying for longer and so fathering more cubs, rather than by improving their probability of survival. Nonetheless, some cub mortality is associated with male takeovers[10,14], caused by the new males killing or excluding some of their predecessors' offspring. Long tenure defers and so reduces this mortality.

We have shown above that larger groups as a whole do better. Figure 2 shows that, assuming equally shared paternity, each individual male in a large group achieves greater expected lifetime's reproductive success (that is, has greater fitness) than he would have achieved in a small group. Comparing the fitness

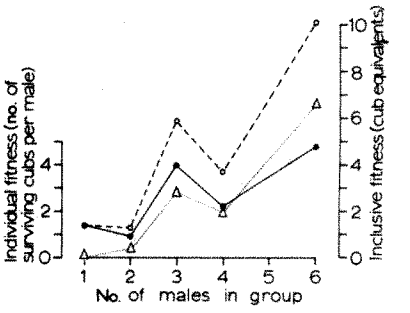


Fig. 2 Individual fitness and inclusive fitness of individual male lions in groups of different sizes. The line for mean individual fitness (●) is derived from Fig. 1*c* assuming equally shared paternity of the cubs born during the males' tenure, and multiplying by the different probabilities of achieving tenure at all (from Table 1). The line for a male's mean inclusive fitness (○) is the sum of first, the number of cubs he has fathered (that is, his mean individual fitness) and second, the number of cubs fathered by his companions multiplied by 0.22 (that is, devalued by their coefficient of relatedness to him). The lowest line (△) shows the inclusive fitness of a male who fathers no cubs of his own.

of males in large groups of 3 or more ($n_1 = 13$ groups) with that of males on their own or in pairs ($n_2 = 12$), the 3.8-fold difference is highly significant ($P = 0.001$, Mann-Whitney U-tests). The advantage does not continue to increase: thus the males in the few (5) very large groups (≥4 males) do not have higher fitness than the males in groups of 3.

The male lions in a coalition are generally relatives: it has been calculated[14] that a male's average degree of relatedness to his companions is 0.22, and that this is almost independent of group size. Cubs fathered by other males in the group also contribute to his inclusive fitness[5,17]. We ignore for present purposes the common increment to inclusive fitness produced by the reproduction of all other relatives everywhere, and consider only the contribution by other members of the male group. It can

generally be assumed that a male forms a coalition with similar-age relatives if he has them. (This was true of 19 out of 21 coalitions whose origins were known; the other two cases were a pair of strangers and a trio of two brothers and a stranger.) The inclusive fitness of each male can thus be calculated, and is illustrated in Fig. 2. There is a marked increase in the inclusive fitness of a male the more related companions he has, such that the inclusive fitness of a male in a large group is 5.7 times that of a lone male or of one of a pair. The initial individual advantage gained through cooperation is amplified through kin selection[5,14,17].

We have assumed in our calculations that paternity (like consortships) is shared equally by all members of a coalition; naturally this is not certain, nor can it be tested in wild populations. However, it can be shown that even if paternity were unequally shared, the inclusive fitness of every member of a large kin-related coalition would still exceed that of a lone male or one of a pair. In the most extreme and improbable case, although a male might sire no cubs of his own, his support of the coalition would enhance the survival of his relatives' cubs. Other things being equal, this would therefore be a better genetic strategy for him than leaving the coalition (Fig. 2).

If members of large groups generally enjoy higher reproductive success, why do not all male lions form large coalitions? It is apparently difficult to form a successful coalition, probably because the bonds necessary have to be developed early in life. Although single unrelated males can sometimes form stable pairs eventually, they seem unable to form or join larger groups, which are intolerant towards strangers. Thus the size of male groups is determined by lion prides' ability to produce and rear male offspring. The small litter size (mean 2.5 cubs[10]), overall even sex ratio[2], and high (~75%) cub mortality[2,10,12] mean that a large male peer group can reliably result only if a considerable number of lionesses in a large pride give birth synchronously. There are indications that prides in fact manage to produce large groups of young males more often than expected (J.D.B. and J.P.H., unpublished data), and how they do so is being investigated. Why they do so is now clear: females who manage to produce large groups of male cubs produce through them disproportionately many grandcubs. Previous cooperation by their mothers (in synchronising births[10] and in rearing cubs cooperatively[2,10]) is a prerequisite for the high lifetime's reproductive success of male lions cooperating in large coalitions.

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Inhibition of mixed lymphocyte response by monoclonal antibody specific for a rat T lymphocyte subset

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Cell-surface differentiation antigens which are associated with particular lymphocyte subsets are likely to be molecules which mediate specific functions of the cells which display them. One way to investigate this hypothesis is to see whether antibodies directed against such antigens will inhibit functional systems. This approach can be attempted with confidence if monoclonal antibodies are used, as these react with one antigenic determinant only and can be used in purified form. In this report we show that a monoclonal antibody which is specific for a subset of rat T lymphocytes inhibits the mixed lymphocyte response (MLR). The antibody acts on the responder cells without killing them.

The W3/25 mouse monoclonal antibody is specific for thymocytes and a subset of T lymphocytes in the rat and the cells in thoracic duct lymph that express the target antigen mediate helper and graft-versus-host functions[1,2]. The W3/25 antigen is relatively sparsely represented on the cell membrane as only about 15,000 molecules of W3/25 antibody are bound at saturation to thymocytes or T cells, yet more than 5×10^5 anti-Thy-1.1 antibodies bind per rat thymocyte[1].

The effect of W3/25 IgG on the MLR is shown in Fig. 1. In these experiments 2×10^5 lymph node cells from PVG/c ($RT1^c$) rats were used as responders to 5×10^5 irradiated spleen cells from the congeneic strain HO.B2 ($RT1^u$) in 0.2 ml cultures as described by Antczak *et al.*[3]. The W3/25 antibody was added to the cells at the start of the culture period and 0.5 μCi of tritiated thymidine (^{3}H-TdR) were added after 72, 96 or 120 h in culture. After a further 18 h the cells were assayed for incorporated label. All assays were carried out in triplicate. Concentrations of W3/25 IgG up to 2.5 ng ml^{-1} in 0.2 ml gave no inhibition but at a concentration of 25 ng ml^{-1} the antibody reduced incorporations of ^{3}H-TdR to a plateau level which was not affected by further increases in antibody concentration. We have observed this inhibition without exception in nine experiments where W3/25 antibody was added to MLRs. In 21 independent observations spread throughout these experiments the mean incorporation of fully inhibited cultures compared with control MLRs was 18.2%, standard deviation 7.3%. Within any one experiment the inhibition became greater, relative to a control MLR, with time up to 120 h. The inhibition was not confined to MLRs with PVG/c versus HO.B2 cells but was also seen in the reverse combination (see also Table 2) and in two other strain combinations which were tested (AO versus DA and PVG/c versus DA).

Other monoclonal antibodies were tested, including W3/13 antibody, which labels all T cells but not B cells[1]; MRC OX 1 antibody, which labels all leukocytes[4] and a monoclonal mouse IgG anti-Thy-1.1 antibody called MRC OX 7 (W. R. McMaster, unpublished) which labels thymocytes but only a small proportion of peripheral T cells in the rat[5]. These antibodies all bind at levels per labelled cell which are at least 2.5 times greater than W3/25 antibody, yet none inhibited the MLR, and W3/13 antibody gave a consistent stimulation of ^{3}H-TdR incorporation (data for W3/25 and W3/13 in Fig. 1; that for MRC OX 1 and MRC OX 7 not shown). For W3/25 and W3/13 antibodies, the extent of binding was checked at 37 °C and greater than 90% saturation was achieved with the same assay conditions used for the MLR at 25 ng ml^{-1} for W3/25 and 5 μg ml^{-1} for W3/13 antibody (D. W. M., unpublished). Furthermore, W3/13 antibody failed to have an inhibitory effect even at a concentration

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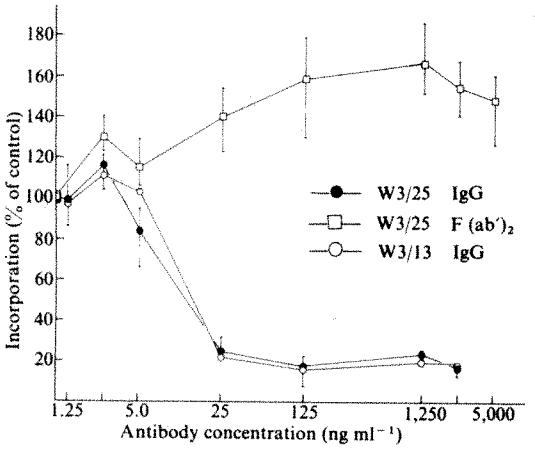


Fig. 1 Effect of W3/25 and W3/13 antibody on the MLR. Data from two independent experiments have been normalised by putting the control MLR incorporation at 100%. The control values were 22×10^3 c.p.m. for the W3/25 experiment and 40×10^3 for the W3/13 experiment. The W3/25 and W3/13 antibodies were added as pure IgG or $F(ab')_2$ prepared by standard procedures. The $F(ab')_2$ contained no IgG detectable by SDS-polyacrylamide gel electrophoresis. The W3/25 IgG contained no myeloma kappa chain since the W3/25 HL hybridoma was used[2]. W3/13 contained some myeloma kappa at a minor level compared with the antibody L chain. Ranges are shown for W3/13 IgG and W3/25 IgG. Ranges for W3/25 $F(ab')_2$ were generally similar to those for W3/25 IgG.

of 50 μg ml^{-1} (data not shown). These observations mean that it is very unlikely that W3/13 antibody did not have an inhibitory effect because it was not used at an adequate concentration.

$F(ab')_2$ fragments prepared from W3/25 IgG also inhibited the MLR and exhibited the same dose–response curve as purified IgG (Fig. 1), indicating that the Fc portion of the antibody molecule is not involved. The serological specificity of the effect was shown by the fact that the inhibition was relieved by absorption of the antibody with rat thymocytes, whereas absorption with rabbit thymocytes had no effect (Table 1).

The effect of W3/25 antibody on responses to T-cell mitogens was investigated by incubating lymph node cells with concanavalin A (Con A) and phytohaemagglutinin (PHA) in the presence of antibody. Cell proliferation caused by these agents was assayed by ^{3}H-TdR incorporation after 48 h in culture and was not significantly inhibited by W3/25 antibody at a concentration as high as 2.5 μg ml^{-1} (Table 1). This is 100 times the level required to give maximal inhibition of the MLR. The potent inhibition of cell proliferation exerted by W3/25 antibody when cells are stimulated by alloantigens does not, therefore, extend to the proliferation induced by T-cell mitogens.

Table 1 Effect of various treatments on the MLR of PVG/c lymph node cells responding to irradiated HO.B_2 spleen cells

Treatment		^{3}H-TdR incorporation (c.p.m. × 10^{-3} per 18-h pulse)
Rat absorption		
Control MLR (2×10^5 cells)		54
+unabsorbed W3/25 antibody		11
+W3/25 absorbed with 6×10^7 rat thymocytes		72
Rabbit absorption		
Control MLR (2×10^5 cells)		52
+unabsorbed W3/25 antibody		11
+W3/25 absorbed with 5×10^7 rabbit thymocytes		9
PHA stimulation		
Control (3.5×10^4 cells)		35
+25 ng per ml W3/25 IgG		37
+2,500 ng per ml W3/25 IgG		32
Con A stimulation		
Control (3.5×10^4 cells)		32
+25 ng per ml W3/25 IgG		58
+2,500 ng per ml W3/25 IgG		31
Control MLR with no preincubation	0 h	38
2×10^5 cells cultured for various times	4 h	61
without addition of antibody before MLR	8 h	73
	24 h	37
	48 h	15
2×10^5 cells cultured for various times	4 h	40
with W3/25 IgG (25 ng ml^{-1}) before MLR	8 h	24
	24 h	63
	48 h	12

In the absorption experiments W3/25 antibody was used in the form of spent tissue culture medium from cultures of cells secreting W3/25 antibody (0.4 μl per culture) while in the other experiments pure W3/25 HL IgG was used. Con A was used at a final concentration of 10 μg ml^{-1} and PHA at 20 μg ml^{-1}.

To discount the possibility that the responding cells in the MLR are killed by the antibody, lymph node cells were cultured without stimulator cells at 37 °C in the presence or absence of W3/25 antibody (25 ng ml^{-1}). They were then washed twice and resuspended in medium lacking antibody and assayed in a standard MLR. These experiments showed that cells could be incubated in the presence of antibody without any reproducible indication that this affected their ability to respond to

Table 2 Alloreactivity of W3/25$^+$ and W3/25$^-$ lymphoid populations

	Incubation period	Responders	Stimulators	^{3}H-TdR incorporation (c.p.m. × 10^{-3} per 18-h pulse)		
				Unstimulated control	MLR	MLR + W3/25 IgG (25 ng ml^{-1})
Expt 1	72 h	2×10^5 W3/25$^+$ (PVG/c)	5×10^5 HO.B2 spleen cells	0.1	57	—
	72 h	2×10^5 W3/25$^-$ (PVG/c)	5×10^5 HO.B2 spleen cells	0.1	7	—
Expt 2	72 h	2×10^5 W3/25$^+$ (PVG/c)	5×10^5 HO.B2 spleen cells	1	17	—
	72 h	2×10^5 W3/25$^-$ (PVG/c)	5×10^5 HO.B2 spleen cells	0.2	4	—
Expt 3	96 h	2×10^5 HO.B2 lymph node cells (unfractionated)	5×10^5 irradiated W3/25$^-$ PVG/c thoracic duct lymphocytes	2	18	3

Each value is the mean of triplicate assays where the range was always < ±15% of the mean. In experiments 1 and 2 MLR's were also done with responders at 1×10^5 and 5×10^5 cells per culture. Typical dose–response curves were obtained and the ratio of W3/25$^+$: W3/25$^-$ responses was similar to that obtained with 2×10^5 responders.

subsequent alloantigenic stimulation. The results of one such experiment are shown in Table 1 with preincubations for various times. At only one time point (8 h) did there appear to be any effect on the subsequent MLR. In another experiment (data not shown) where cells were preincubated for 24 or 48 h no inhibition was observed.

These findings show that W3/25 antibody inhibits the MLR quite specifically without killing the cells. This effect prompts questions about the role of W3/25 positive cells in the MLR. It might be expected that the major part of the cell proliferation in the MLR is due to W3/25 positive cells and that in conditions of inhibition by W3/25 antibody these cells have no role as responders. This possibility was investigated using a fluorescence activated cell sorter to prepare populations of W3/25-positive and -negative cells from thoracic duct lymph[2]. In two independent experiments, covering a range of responding cell doses, and culture periods of 72 and 96 h, the incorporation of ^{3}H-TdR by W3/25-positive cells was found to be at least four times that of the W3/25-negative cells when equal numbers of responding cells were compared (Table 2).

In a third experiment, stimulator cells were depleted of W3/25-positive T cells in the cell sorter to a level of 0.8% contamination and used to stimulate fresh lymph node cells. The W3/25-negative cells acted efficiently as stimulators (Table 2) and the resultant MLR could be inhibited by W3/25 antibody giving the same dose–response curve as that shown in Fig. 1 (incorporation by culture treated with 25 ng ml^{-1} W3/25 IgG = 19.3% of control MLR). Similar results were obtained in another experiment. It might be argued that the stimulator effect in these experiments was dependent on very small numbers of W3/25-positive contaminating cells. However, this is unlikely as although W3/25-positive cells were able to stimulate an MLR, they were no more potent than W3/25-negative cells and when added at 2×10^4 cells per culture gave no detectable stimulation of the MLR (M. W., unpublished). This number of W3/25-positive cells is five times greater than the estimated number of contaminating cells present in the W3/25-negative stimulators used in experiment 3 of Table 2.

These results suggest that the targets of the antibody are the responding cells rather than the stimulating cells, and the inhibition data indicate a blocking function for added W3/25 antibody. It is thus possible that the W3/25 molecule is of functional significance in the MLR response.

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Lymphocytotoxic antibodies produced by H–2 allo-immunisation distinguish between MuLV-positive and -negative substrains of the same H-2 haplotype

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In C57BL mice of certain H–2 types, such as B10.A (H–2^a), ecotropic leukemia virus has been shown to be transmitted by milk-borne infection. By selecting virus-positive and virus-negative B10.A mothers and foster-nursing the B10 (H–2^b) newborn animals, four sub-lines of C57BL/10 mice have been obtained—B10.A V$^+$, B10.A V$^-$, B10 V$^+$ and B10 V$^-$ (see Fig. 1 and ref. 1). Milk transmission of naturally prevalent B-tropic virus (V$^+$ sub-lines) leads to a persistent infection of all the offspring over more than 8 generations[2]. We have studied the consequences of this kind of virus infection on the expression and immunogenicity of antigens coded for by the H–2 complex. This complex exerts a marked influence on oncogenesis by a variety of oncogenic RNA viruses[3,4]. The H–2K and H–2D loci restrict the specificity of virus-specific cytotoxic T lymphocytes[5]. In some instances, a direct physical association between H–2 and viral antigens has been demonstrated[6–10]. In our work, groups of B10.A V$^+$ and B10.A V$^-$ mice were immunised with B10 V$^+$ and B10 V$^-$ lymphoid cells. Production of anti-H–2^b antibodies was expected and observed in these sera. However, surprisingly, in the combination of B10 V$^+$ donor and B10.A V$^-$ recipient, after prolonged hyperimmunisation, we obtained sera that reacted positively in the routine microlymphocytotoxicity test with B10.A V$^+$ lymph-node cells, but not with B10.A V$^-$ cells. B10 V$^-$ cells were able to absorb this antibody. Our preliminary conclusion is that the milk-transmitted virus, soon after birth, introduces a serologically detectable antigen which is recognised in association with H–2. The prerequisite for the formation of this kind of antibody is the immunisation of H–2 haplotype-different V$^-$ recipients with V$^+$ donor cells.

The derivation of sub-lines of C57BL/10 (B10) mice with (V$^+$) and without (V$^-$) milk-transmitted B-tropic virus is schematically presented in Fig. 1. The individual mice used in the experiments were from the 10th to 12th generation of the respective lines after their original preparation. For immunisations, donor spleen + thymus + lymph-node cells (in 'doses' of 1 donor per 10 recipients) were injected intraperitoneally at weekly intervals with a 1-week interval after every fourth injection. Serum samples were collected every week, 3–4 days after injection or 3–9 days after the last immunisation[11]. The routine microlymphocytotoxicity test on mouse lymph-node cells (or spleen cells) was carried out in serial geometrical dilutions[11]. Immunofluorescence tests were carried out using a fluorescein-labelled rabbit anti-mouse IgG serum, prepared in our Institute. Antibodies against AKR type viral-envelope antigens (VEA) were measured by a radioimmunoprecipitation (RIP) test[2].

The results of four immunisations, carried out in the combination of donor B10, recipient B10.A, are listed in Table 1. All sera exhibited an almost equally strong cytotoxic reaction on cells of the B10 V$^+$ and B10 V$^-$ substrains. An unexpectedly strong cytotoxic reaction was observed with serum CLS-12 (B10 V$^+$ donor, B10.A V$^-$ recipient) with the lymph-node (or spleen) cells of B10.A V$^+$ mice. More than 20 randomly chosen individual mice of the B10.A V$^+$ and B10.A V$^-$ substrains, of both sexes, 1–10 months old, were tested. The result was always the same; the individuals V$^+$ reacted positively whereas the

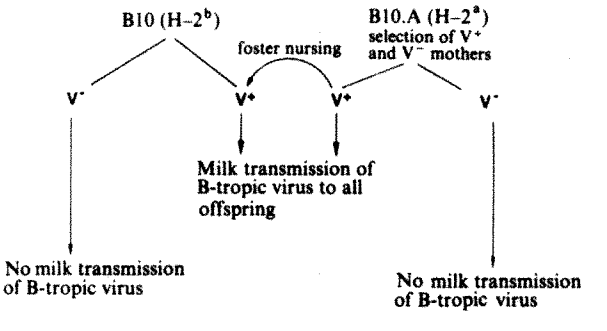


Fig. 1 Derivation of V$^+$ and V$^-$ sub-lines (see refs 1, 2).

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Table 1 Reaction pattern of sera from bleedings during four anti-H-2^b allo-immunisations (CLS-10, -11, -12, -13)

Serum no.	Donor strain and H-2	Recipient strain and H-2	Serum samples tested	Microlymphocytotoxicity titre on cells: B10 (bb)*	B10.A(2R) (kb)	B10.A (5R) (bd)	B10.BR (kk)	B10.A V$^+$ (kd)	B10.A V$^-$ (kd)
CLS-10	B10 V$^-$ H-2^b	B10.A V$^-$ H-2^a	B to Q†	64‡	64	64	0	0	0
CLS-11	B10 V$^-$ H-2^b	B10.A V$^+$ H-2^a	B to Q	64	64	64	0	0	0
CLS-12	B10 V$^+$ H-2^b	B10.A V$^-$ H-2^a	B (2)	64	64	64	0	0	0
			C (3)	64	64	64	0	0	0
			D (4)	64	64	64	0	0	0
			F (6)	64	64	64	0	0	0
			G (7)	64	64	64	0	0	0
			H (8)	64	64	64	0	0	0
			I (9)	64	64	64	0	0	0
			J (10)	64	64	64	0	0	0
			K (11)	64	64	64	0	0	0
			L (12)	64	64	64	0	16	0
			M (13)	64	64	64	0	64	0
			N (14)	64	64	64	0	16	0
			O (15)	64	64	64	0	64	0
			Q (17)	64	64	64	0	16	0
CLS-13	B10 V$^+$ H-2^b	B10.A V$^+$ H-2^a	A to Q	64	64	64	0	0	0

* Strain designation and H-2 alleles at the K and D locus.
† Letters B to Q designate the serial serum sample; the number in parentheses shows the week of immunisation.
‡ Sera were only tested in six dilutions. All reactions with strains B10 V$^+$, B10 V$^-$, B10.A (2R) and B10.A (5R) were still strongly positive at dilution 1:64.

reaction with V$^-$ individuals was negative. Hence, with the routine microlymphocytotoxicity test, simple 'serotyping' of the B10.A V$^+$–B10.A V$^-$ difference became possible.

The anti-V$^+$ activity in the CLS-12 combination developed after about 12 weeks of allo-immunisation. None of the CLS-10, CLS-11 or CLS-13 sera, prepared and tested in parallel, exhibited this kind of activity (Table 1). The data in Table 1 were obtained by testing serum pools obtained from four to six mice from the immunised groups. Because we have shown previously[11,12] that individual mice of one inbred strain differ widely in their ability to produce anti-H-2 antibodies, the serum samples taken during the last two weeks of immunisation (R+S weeks) were tested as samples of individual mice, not as serum pools. The reactions of the individual CLS-12 sera (10 mice) are shown in Table 2. Of 10 sera, 7 react strongly with B10.A V$^+$ cells, but not at all with B10.A V$^-$ cells. Two sera exhibited a titre of 1:1,024 with B10.A V$^+$ cells.

Absorption experiments (Table 3) have shown that both B10 V$^+$ and B10 V$^-$ cells absorbed all cytotoxic activity from CLS-12 sera, and that with B10.A V$^+$ and control B10.A V$^-$ cells, V$^+$ cells completely removed activity only against themselves, but left strong activity against the haplotype-specific H-2^b antigens of the donor strain. B10.A V$^-$ cells absorbed neither anti-H-2^b nor anti-B10.A V$^+$ activity. The observation that B10 V$^-$ cells absorbed the anti-B10.A V$^+$ activity, whereas B10.A V$^-$ cells did not, indicates that MuLV antibodies are not responsible for the reactivity of the CLS-12 sera with B10.A V$^+$ cells. First, B10 V$^-$ cells are virus negative. Second, B10.A V$^-$ cells are virologically identical to B10 V$^-$ cells (virus negative) but they do not absorb the activity against B10.A V$^+$ lymphocytes. Two other sets of data support the conclusion that anti-MuLV antibodies are not responsible for the anti-B10.A V$^+$ activity. Two CLS-12 sera positive with B10.A V$^+$ mice were tested in the RIP assay, by far the most sensitive assay for detecting antiviral envelope antibodies, but no such antibodies were found. Moreover, we tested several mouse sera with high titres of antibodies to viral envelope antigens but none of them lysed B10.A V$^+$ lymph-node cells. Two of these sera were from 3-month-old B10.A V$^+$ mice with high levels of anti-VEA antibodies. Finally, 20 B10.A V$^-$ mice were immunised with B10.A V$^+$ cells (by an immunisation schedule identical to that applied for the preparation of the CLS-12 sera), but none of these sera became cytotoxic for B10.A V$^+$ (B10 V$^+$) lymph-node cells. The tests were carried out after 8, 9, 18 and 20 weeks of immunisation; in comparison (Table 1), CLS-12 sera became positive after 12 weeks of immunisation. This finding shows that only allogeneic V$^+$ cells are able to induce cytotoxic anti-B10.A V$^+$ antibodies in B10.A V$^-$ recipients.

The results described above show a clear-cut difference between the B10.A V$^+$ and B10.A V$^-$ substrains detected by the lymphocytotoxicity test on normal cells from lymph-node or spleen. These two strains differ only in the presence of a milk-transmitted MuLV, the trait for which they were selected 10–12 generations previously. The serologically defined differences between the two strains might be based on either of two mechanisms. First, a gain mutation might have become fixed in the V$^+$ strain and have caused the appearance of a new specificity common to H-2^b and the new H-2 haplotype of B10.A V$^+$. Alternatively, a loss mutation in the B10.A V$^-$ strain would allow the production of antibodies against a common antigen shared by the H-2^b and H-2^a haplotypes. Both possibilities seem unlikely for two reasons: (1) the anti-B10.A V$^+$ antibodies occurred only in the combination defined as CLS-12 (Table 1), and (2) skin grafts survived between the B10.A V$^+$ and B10.A V$^-$ substrains (data not shown). The second possibility is that the two substrains still differ only in the original trait for which they were selected[1], that is, positive or negative for the milk-transmitted ecotropic MuLV. If this were so, embryos and newborn animals of the B10.A V$^+$ substrain should not react with CLS-12 sera, and the reactivity should develop only after drinking mother's milk.

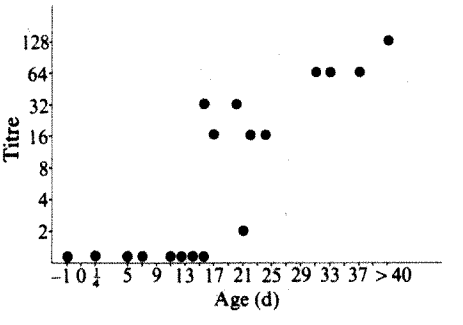


Fig. 2 Postnatal development of positivity with CLS-12 serum (no. 10, R+S bleeding) on spleen cells of B10.A V$^+$ mice. The mothers of all litters examined reacted positively (titre 1:64–128). On 1 day before and after birth, the immunofluorescence test was used; on further days, the microcytotoxicity test on spleen cells was used. Titres represent the highest serum dilution with more than 80% killed cells.

The second possiblity was examined by testing spleen cells of B10.A V$^+$ embryos and newborn mice with CLS-12 serum using immunofluorescence and cytotoxicity tests. Embryos, 1 day before birth, and newborn mice that were maintained 6 ± 3 h after birth without mother's milk, did not react with CLS-12 sera. The cytotoxicity test with CLS-12 sera became positive on spleen cells after day 45 (Fig. 2).

The antibodies in B10.A V$^-$ (H-2^a) anti-H-2^b sera which recognise the difference between B10.A V$^+$ and B10.A V$^-$ sublines of H-2^a mice, have the following characteristics: (1) anti-B10.A V$^+$ activity only occurred after immunisation of B10.A V$^-$ recipients with B10 V$^+$ cells. Such antibodies were not detectable after immunisation with B10 V$^-$ cells or after immunisation of B10.A V$^+$ recipients. (2) The anti-B10.A V$^+$ activity appeared only very late—after more than 10 weeks of allo-immunisation. (3) B10 V$^+$ and B10 V$^-$ cells each absorbed all activity. (4) B10.A V$^+$ cells absorbed only that part of the activity which was directed against the B10.A V$^+$ cells, whereas the anti-B10 V$^+$ and anti-B10 V$^-$ activity remained virtually unchanged. (5) B10.A V$^-$ cells absorbed neither the anti-H-2^b nor the anti-B10.A V$^+$ activity. (6) The target structure on the B10.A V$^+$ cells, responsible for the reaction with CLS-12 sera, developed postnatally, probably as a consequence of virus transmission through milk or placental infection. This target structure develops much later than do H-2 antigens, which are already detectable at birth by cytotoxicity and reach adult sensitivity within 1-3 days[13].

These characteristics cannot be explained by assuming that the anti-V$^+$ and anti-H-2^b activities in CLS-12 sera are due to completely separate populations of antibodies. In that case, B10 V$^-$ cells would not be expected to absorb the anti-B10.A V$^+$ activity, unless one assumes that B10 V$^-$ mice also have the V$^+$ antigen but in a form that only absorbs and is not immunogeneic. This possiblity seems far fetched. Indeed, B10 V$^-$ mice express little or no viral structural proteins despite the presence in their genome of latent DNA pro-viruses[1,2,14,15]. Moreover, B10.A V$^-$ cells, that do not differ from B10 V$^-$ cells in the lack of expression of infectious virus and/or viral structural proteins[1,2], do not absorb the activity against B10.A V$^+$. Therefore, it seems likely that all cytotoxic activity of CLS-12 sera is due to anti-H-2^b antibodies, including the anti-B10.A V$^+$ activity. On the other hand, the antibody activity against B10.A V$^+$ cells constitutes only minor fraction of the anti-H-2^b sera, because after absorption with B10.A V$^+$ cells, all activity against B10.A V$^+$ is removed, whereas strong anti-H-2^b activity remains.

Having considered all these points, we propose the following explanation for the current observation. Milk-transmitted MuLV induces a lymphocyte antigen (V$^+$) which in association with allogeneic H-2^b structures becomes immunogenic in the late phase of B10.A V$^-$/anti-B10 V$^+$ immunisation. The resulting antiserum cross-reacts with an antigen on normal B10.A V$^+$ lymph-node cells (and spleen cells) in the microlymphocytotoxicity test. The target antigen on B10.A V$^+$ lymphocytes develops within a few days after birth. Whether physical association between V$^+$ and H-2 structures is necessary for immunogenicity remains unclear. The observation that antibody against this antigen was only produced after allo-immunisation in the combination of B10 V$^+$ donor, B10.A V$^-$ recipient, as well as the finding that B10 V$^-$ absorbed all antibody activity, suggests that the immunogen is intimately related to H-2 and possibly represents a virus-altered H-2 antigen.

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Table 2 Reaction pattern of 10 individual anti-H-2^b (CLS-12) sera

Individual recipients mouse no.	Microlymphocytotoxicity titre on cells* B10A V$^+$	B10.A V$^-$
1	8	0
2	16	0
3	64	0
4	0	0
5	64	0
6	4	0
7	1,024	0
8	1,024	0
9	2	0
10	64	0

The serum samples from individual mice were pools obtained from bleedings on the R(18th) and S(19th) weeks of immunisation.
* All sera had a titre > 1 : 1,024 on B10 V$^+$ and B10 V$^-$ cells.

Table 3 Absorption experiments with CLS-12 (B10 V$^+$ donor, B10.A V$^-$ recipient) sera

Serum designation	Cells used for absorption	Dose	Cells tested after absorption B10 V$^+$	B10 V$^-$	B10.A V$^+$	B10.A V$^-$
CLS-12-S-8	None		1,024	1,024	1,024	0
	B10 V$^+$	100×10^6	0	0	0	0
	B10 V$^-$	100×10^6	0	0	0	0
CLS-12-R-7	B10.A V$^+$	100×10^6	1,024	0	0	0
	B10.A V$^-$	100×10^6	1,024	1,024	1,024	0

Identical results were obtained with two other CLS-12 sera. 50 μl of serum CLS-12 were absorbed with spleen + lymph-node cells.

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Diethylcarbamazine enhances antibody-mediated cellular adherence to *Brugia malayi* microfilariae

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Treatment with the antifilarial drug diethylcarbamazine (DEC) results in a rapid decline in the number of microfilariae circulating in the blood of infected hosts[1]. DEC induces morphological changes in the surface layers of microfilariae, but these alterations alone are probably insufficient to cause the death of the parasite, because the drug fails to reduce microfilaraemia in animals lacking filarial antibodies, and also does not shorten the survival of microfilariae *in vitro*[1-4]. The effect of DEC *in vivo* is thought to result from the trapping of microfilariae in the liver, where they undergo lysis[1,2,5,6].

Peripheral blood leukocytes from uninfected donors can be made to adhere to microfilariae of *Brugia malayi in vitro*; cellular adherence is promoted by antibodies reacting with the microfilarial sheath, or by a heat labile factor present in normal serum (thought to be complement). The adherence promoting antibody is probably of the IgG class because: (1) only patients' sera that contain high titres of IgG antisheath antibodies

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Table 1 Enhancement of antibody-mediated cellular adherence to *B. malayi* microfilariae by diethylcarbamazine (DEC)

DEC concentration ($\mu g\ ml^{-1}$)	% Microfilariae with ≥5 cells attached				
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
0	25±2	30±3	15±2	34±4	24±3
100	*48±6*	*59±8*	*35±5*	*90±8*	*50±6*
10	*43±5*	*51±6*	*32±4*	*70±5*	*50±5*
5	*38±4*	*42±4*	*32±5*	*67±6*	—
1	29±4	41±2	*30±3*	*56±5*	*38±4*
0.5	26±2	*32±3*	*25±3*	*48±5*	—
0.1	24±3	*30±4*	*23±2*	*46±4*	*32±4*

Results shown are means of triplicate determinations ± s.d. Numbers in italics are significantly different from control values ($P < 0.01$).

Table 3 DEC increases cellular adherence to microfilariae mediated by sera that are inactive in the absence of the drug

DEC concentration ($\mu g\ ml^{-1}$)	%Microfilariae with ≥5 cells attached in presence of serum			
	No. 5012	No. 5071	No. 5127	No. 4075
0	3±1	10±2	2±1	7±1
100	*34±3*	*27±3*	2±1	8±1
10	*48±5*	*18±5*	1±1	8±2

Sera from human patients with Malayan filariasis were used at a dilution of 1:4. Results shown are means ± s.d. of triplicate determinations. Numbers in italics are significantly different from control values ($P < 0.05$).

detected by indirect immunofluorescence are active in this regard; (2) the adherence reaction is reversed by the addition of low concentrations of staphylococcal protein A which binds to the Fc portion of IgG (unpublished observation); and (3) purified IgG antisheath antibody promotes similar adherence of cells to microfilariae of *Wuchereria bancrofti*[7]. To investigate further the mechanism of action of DEC, we examined the effect of this drug on the adherence reaction. The results indicate that DEC enhances antibody-, but not complement-mediated adherence of buffy coat cells from normal donors to *B. malayi* microfilariae. The effect results from a direct action of the drug on microfilariae and not from a functional activation of leukocytes.

The adherence assay was carried out by sequentially adding the following constituents, each in 0.05 ml volumes to triplicate wells of round-bottom microtitre plates: microfilariae ($4{,}000\ ml^{-1}$); diluted antiserum (see below), fresh human serum diluted 1:4 and/or culture medium (Hanks' minimal essential medium, MEM); and buffy coat cells (2×10^5, prepared by dextran sedimentation of blood from normal volunteers). Serum from three different subjects with elephantiasis due to *B. malayi* was used as the source of IgG microfilarial antibody; these sera were diluted with MEM to promote cellular adherence in the absence of DEC to less than 35% test microfilariae. DEC was dissolved in MEM; the *p*H of the solution was adjusted to 7.4 with bicarbonate buffer. Adherence was scored after 2 h of incubation at 37 °C.

DEC increased the proportion of microfilariae that formed rosettes with buffy coat leukocytes in the presence of antisheath antibodies in a dose-dependent fashion. The results of five representative experiments in which a wide range of drug concentrations was tested simultaneously are shown in Table 1.

The number of adhering cells per worm was also increased in the presence of DEC, but this phenomenon was difficult to quantitate. In the absence of DEC, 5–20 cells were attached to the surface of microfilariae; in the presence of the drug, most worms were surrounded by several concentric layers of cells, generally exceeding 50 in number. No adverse effects on the motility or viability of microfilariae were observed during the short (2 h) culture period. Microfilariae of *W. bancrofti* are killed under similar experimental conditions, but only after longer periods of incubation[7].

In preparations stained with Wright–Giemsa, lymphocytes, neutrophils, eosinophils and monocytes were seen to adhere to microfilariae; DEC did not selectively increase the adherence of one or another cell type to the worms.

To determine whether the increased cellular adherence resulted from an effect of the drug on the microfilariae, on the leukocytes or on both, experiments were done in which microfilariae or buffy coat cells were incubated separately with DEC and then washed extensively before they were used in the adherence assay. Preincubation of leukocytes with the drug did not increase cellular adherence to microfilariae. In contrast, preincubation of microfilariae markedly enhanced the proportion of worm-cell rosettes formed in the presence of antibody (Table 2). In seven experiments, complement-mediated adherence of buffy coat cells to microfilariae was not enhanced by DEC added in final concentrations of up to 250 $\mu g\ ml^{-1}$. Similarly, DEC did not increase the proportion of worms forming rosettes with cells in serum-free medium.

These results clearly indicate that diethylcarbamazine increases the adherence of leukocytes to *B. malayi* microfilariae mediated by antibodies reacting with the surface of the worms. The effect appears to result from a direct action of the drug on microfilariae. However, drug-induced surface alterations *per se* do not promote cellular adherence *in vitro* in the absence of microfilarial antibodies. Likewise, such changes do not promote the *in vivo* hepatic trapping of microfilariae in experimental animals unless antibodies are also present in the treated host[4]. Treatment with DEC of human subjects with patent microfilaraemia rapidly reduces the number of circulating microfilariae. Yet, the serum of such patients contains no antisheath antibodies detectable by indirect immunofluorescence. There are several possible explanations for this apparent discrepancy: it is possible that antisheath antibody titres in microfilaraemia are too low to be detected by rather insensitive immunofluorescent methods; such antibodies may already be bound to the parasites and therefore not readily detectable in serum; or DEC may act *in vivo* by several mechanisms of which only one is measured by the *in vitro* assay used for these studies. In addition, our findings are also consistent with the hypothesis that DEC may act by rendering accessible previously hidden antigenic determinants, antibodies to which might not be detectable in immunofluorescent assays using non drug-treated microfilariae. The observation that some sera from *B. malayi* infected donors fail to promote cellular adherence to microfilariae in the absence of DEC, but enhance adherence in the presence of the drug (Table 3) supports this hypothesis. The mechanism by which DEC unmasks parasite antigens and the exact nature of these antigenic determinants remain to be determined.

We thank Dr J. B. Johnson of Lederle Laboratories for supplying diethylcarbamazine (Hetrazan). These studies were supported by a grant from the Rockefeller Foundation.

Table 2 Enhanced antibody-mediated cellular adherence following pretreatment of microfilariae with DEC

DEC concentration ($\mu g\ ml^{-1}$)	% Microfilariae with ≥5 cells attached	
	Worms pretreated	Cells pretreated
0	27±3	27±3
100	*40±4*	25±3
10	*37±4*	31±4
1	33±3	29±2

Microfilariae (20,000) or buffy coat cells (5×10^6) were incubated with the drug concentrations shown for 30 min at 37 °C and washed three times before the addition of leukocytes. Numbers in italics are significantly different from control values ($P < 0.01$). Each datum is the mean ± s.d. of triplicate determinations.

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Genomic rearrangements correlated with antigenic variation in *Trypanosoma brucei*

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The capacity of African trypanosomes to express sequentially a large repertoire of different surface antigens during an infection[1–3] enables the parasite to evade the immune response of its host, and makes attempts to produce a vaccine against the disease difficult. It is evident that point mutations cannot account for antigen diversity[4,5]. Variable antigens like immunoglobulins are derived from an extensive family of genes of which only one is expressed in a given cell. As somatic recombination is involved in the immunoglobulin gene system[6], this similarity prompted us to search for somatic rearrangements in trypanosome variable antigen genes. We have constructed a recombinant plasmid containing approximately half the DNA sequence coding for a *Trypanosoma brucei* variable antigen and hybridised the inserted sequences to various restriction enzyme digests of nuclear DNA from different trypanosome clones. Differences in the sizes of restriction fragments hybridising to the inserted variable antigen coding sequence show altered positions of enzyme sites relative to this sequence, indicating different arrangements of DNA sequences around this gene in different trypanosome clones.

Three clones of *T. brucei* expressing different variable antigens were derived from stock LUMP 227 (UHEMBO/64/EATRO/795). Clone A was derived from a relapse infection of clone C. Clone B was derived from a relapse infection of clone A. Clone X was derived from a different trypanosome stock, S427. All trypanosome populations, grown in lethally irradiated rats for these studies, were monitored for variable antigen type homogeneity by immunofluorescent techniques[7].

Messenger RNA was prepared from *T. brucei* clone A, expressing variable antigen ILTat 1.2, as described previously[8]. A preparation enriched for variable antigen mRNA was obtained by two cycles of density gradient centrifugation. Double-stranded cDNA was made on this template by a slight modification of the procedure of Wickens *et al.*[9], and inserted into the plasmid vector pBR322 by the G–C boundary method[10]. Transformed bacteria containing recombinant plasmids were screened by *in situ* filter hybridisation[11]. Isolated plasmids were further screened for variable antigen coding sequences by hybrid-arrested translation[12]. Thus it was shown that plasmid pcB602 contains variable antigen coding sequences (Figs 1 and 2).

A filter hybridisation of the inserted sequences of pcB602 to *Hin*dIII fragments of nuclear DNA from four different *T. brucei* clones is shown in Fig. 3. In all clones there is a strongly hybridising fragment. It is the same size in clones B and C (4.5 kilobases) but slightly larger (5.0 kilobases) in clone A, this clone expressing the sequences in the probe. In clones B and C

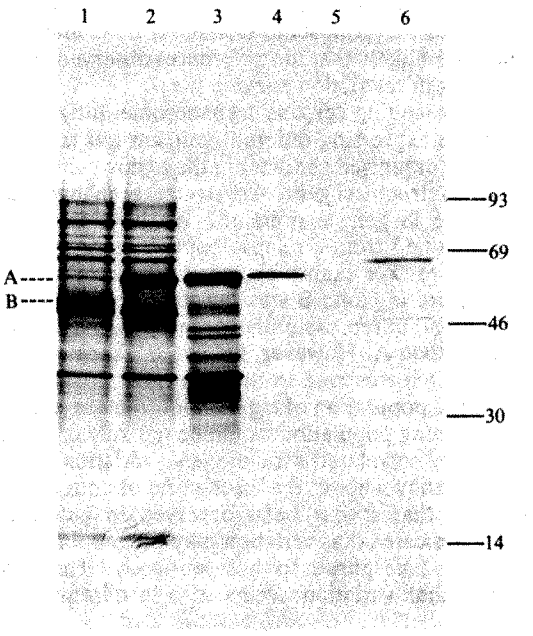


Fig. 1 Identification of variable antigen band in SDS polyacrylamide gel electrophoresis of *in vitro* translation products. Translation products of total mRNA from *T. brucei* clones: lane 1, clone B; lane 2, clone A; lane 3, translation products of partially enriched variable antigen mRNA from clone A. Translation products of total mRNA from clone A immunoprecipitated with: lane 4, antiserum against clone A antigen; lane 5, with normal rabbit serum. Lane 6, immunoprecipitate of variable antigen from *in vivo* labelled trypanosome with anti-clone A variable antigen antiserum. Antiserum was raised against purified variable antigen[20] by conventional procedures. Polyadenylated RNA was further purified by two cycles of sucrose density gradient centrifugation. Translation products were made in the rabbit reticulocyte cell-free system[21]. Solubilised extracts of ^{35}S-methionine-labelled proteins from the cell free translation reactions and from trypanosomes labelled *in vivo* (S. Z. Shapiro, unpublished) were made by the procedure of Shapiro and August[22] and immunoprecipitated by the proceure of Kessler[23]. Electrophoresis was performed with a 7.5–17.5% polyacrylamide gradient slab gel containing 0.1% SDS using the discontinuous buffer system of Maizel[24]. Position of *in vitro* synthesised variable antigen bands are indicated at A (clone A) and at B (clone B). The former is identified by immunoprecipitation. It has a lower apparent molecular weight (60,000) than the glycosylated *in vivo* labelled protein (63,000) as expected[8].

there is a less strongly hybridising fragment of very different size in each clone, 7 kilobases in clone B and 8.5 kilobases in clone C. In clone A there is weaker hybridisation to three fragments, 11, 8 and 6 kilobases long.

Plasmid pcB602 has been sequenced (K. Chen, J. E. Donelson and R.O.W., unpublished work). It contains 902 base pairs of coding sequence. There is a single *Hin*dIII site which is 293 base pairs from one end. If the same site were present in genomic DNA we would expect to find at least two genomic *Hin*dIII fragments hybridising to the two segments of the probe sequence. This would be consistent with what is seen with clones B and C. With clone A there are three weakly hybridising fragments. This could result from the presence of *Hin*dIII sites in very long intervening sequences, or asymmetric length heterogeneity of multiple gene copies. There is no evidence concerning gene reiteration.

Using nuclear DNA from clone X, some hybridisation to a fragment of very different size from the major fragments observed in the other clones (13 kilobases) was detected. This result shows that this clone contains a genomic sequence of sufficient homology to the clone A antigen coding sequence to hybridise in these conditions. In similar experiments using genomic DNAs digested with the restriction enzymes *Hin*cII,

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*Hin*fI and *Xho*I differences in hybridisation patterns were observed between trypanosome clones A, B, C and X in all cases. Thus it is unlikely that the differences observed are due to point mutations in restriction enzyme sites.

The sequence coding for one trypanosome antigen is conserved in clones expressing different antigens but these clones have differently organised genomes in the region containing the clone A antigen structural gene. Are structural changes responsible for change in gene expression? We have found that a trypanosome cloned from a relapse population has an altered genomic structure. We cannot exclude the possibility that the genomic structure in clone B was selected fortuitously by the cloning of a single unrepresentative organism from the relapse population of clone A. However, neither weaker nor stronger bands in clone B correspond to those in clone A. The cloning from the relapse population of a genomic structure not detectable in the infecting population would be unlikely unless structural changes are correlated with antigenic variation.

Very little is known about the mechanism of antigenic variation. The fact that it has been observed in cultured trypanosomes[13] indicates that variation may occur spontaneously rather than as a response to host antibody. The simplest assumption is that variation occurs at a low frequency in a trypanosome population, and that new variants become apparent in successive waves of parasitaemia as the preceding ones are eliminated by the host's immune system[3]. If this is an accurate interpretation, then we are observing an alteration of gene expression which is stably inherited by the vast majority of progeny cells. The process resembles differentiation in higher eukaryotes more than transient forms of control of gene expression, such as hormonal regulation. Thus antigenic variation may provide an important model for some general processes in eukaryotic differentiation.

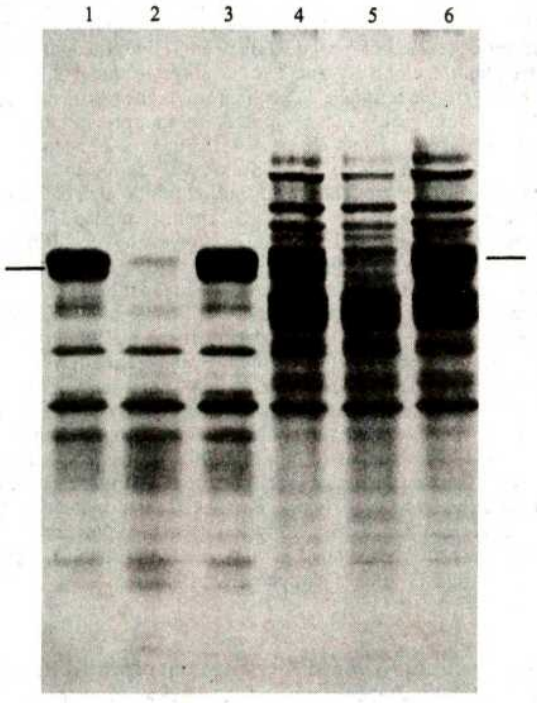


Fig. 2 Hybrid-arrested translation confirming that pcB602 contains variable antigen coding sequences. Translation products of partially enriched variable antigen mRNA; lane 1, unhybridised; lane 2, hybridised with pcB602; lane 3, hybridised with pcB602 then denatured by boiling. Translation products of total mRNA: lane 4, unhybridised; lane 5, hybridised with pcB602; lane 6, hybridised with pcB602 then denatured by boiling. Hybridisations, carried out according to Paterson[12], contained in a final volume of 50 μl, 10 μg of *Hae*III-digested pcB602 and 1 μg of enriched mRNA fraction or 2 μg *Hae*III-digested pcB602 and 1 μg total mRNA. After 4 h at 48 °C, 100 μl of cold water was added and the mixture was divided. One aliquot was boiled for 90 s, before both were ethanol precipitated and translated in the rabbit reticulocyte system. Electrophoresis of translation products was through a 10% polyacrylamide SDS slab gel. The variable antigen band as identified by immunoprecipitation is marked by the horizontal bars. Specific and reversible inhibition of synthesis of this polypeptide by hybridisation with pcB602 shows that the plasmid contains variable antigen coding sequences. Control experiments with pBR322 and another recombinant plasmid containing G–C boundaries gave no inhibition of synthesis of this band.

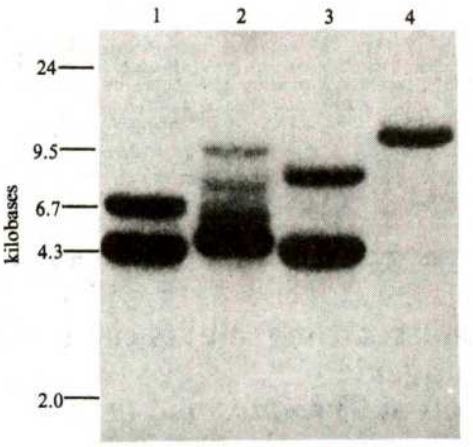


Fig. 3 Hybridisation of inserted sequences of pcB602 to *Hin*dIII fragments of nuclear DNA: lane 1, clone C; lane 2, clone A; lane 3, clone B; lane 4, clone X. Plasmid pcB602 was digested with *Pst*I. The three fragments of the inserted sequence were separated by electrophoresis, recovered from an agarose gel[25] and labelled with ^{32}P by nick translation[26] to high specific activity ($\geq 250 \times 10^6$ ^{32}P c.p.m. μg^{-1}). Nuclear DNAs were prepared from cloned trypanosomes using a modification of published procedures[27]. DNA was completely digested with the restriction enzyme *Hin*dIII and electrophoresed in 0.6% agarose gels. Fragments were transferred to nitrocellulose filters by the blotting procedure of Southern[28], and hybridised to the nick translated probe (in 120 mH Tris HCl, *p*H 7.4, 4 mM EDTA, 0.6 M NaCl, 0.1% sodium pyrophosphate, 0.1% SDS, 0.2% each of Ficoll 400, polyvinyl pyrollidone and bovine serum albumin, and 100 μg ml^{-1} sonicated denatured calf thymus DNA) at 68 °C for 60 h. After extensive washing the filters were autoradiographed using Kodak XR5 film with intensifying screens.

Our data do not allow us to distinguish between the many possible forms of DNA rearrangement that could account for the changes observed. Immunoglobulin genes are known to be constructed by movement of variable region genes into proximity with the constant region gene[6]. While trypanosome variable antigens do exhibit general immunological cross-reactivity[14], it has not yet been determined whether this involves regions of polypeptide homology or the attached carbohydrate structures (A. F. Barbet, personal communication). Therefore, we cannot implicate an analogous mechanism in construction of variable antigen genes. In prokaryotes translocatable genomic elements are known to exert control over gene expression[15–17]. Somewhat analogous translocating element hypotheses have been used for eukaryotes to explain some phenomena in the genetics of maize[18] and *Drosophila*[19]. Similar mechanisms might possibly occur in trypanosomes.

We have observed differences in genomic structure in the region containing sequences coding for the clone A variable antigen in different clones of *T. brucei* expressing this and two other antigens. We propose that the rearrangements observed may be involved in controlling the expression of this gene. A sequence with some homology to that coding for clone A variable antigen is also present in a clone from *T. brucei* stock S427.

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in containment conditions specified by the NIH Guidelines for recombinant DNA research and under the supervision of the ILRAD Biohazards Committee.

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Regulation of lymphocyte mitogenesis by (2′–5′) oligo-isoadenylate

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Interferon has been reported to affect cell proliferation in various tumour and normal cell cultures[1]. In human cells, the anti-growth effect of interferon is under the control of chromosome 21, like the antiviral effect[2,3], and the two activities appear to reside in the same glycoprotein[4]. In synchronised fibroblast cultures interferon inhibited DNA synthesis by blocking initiations of DNA replication[5,6]. Another system in which the inhibitory effect of interferon on DNA synthesis was particularly clear is lymphocyte mitogenesis. In lymphocytes stimulated by lectin mitogens or allogeneic cells, interferon inhibits DNA synthesis, and the RNA and protein synthesis observed before the S phase of the cell cycle[7–9]. In interferon-treated cells, several enzymes are induced, including the (2′–5′) oligo-isoadenylate [oligo(A)] synthetase E, which polymerises ATP into a series of oligonucleotides of general structure ppp(A2′p)$_n$5′A (refs 10–12). The (2′–5′) oligonucleotides inhibit protein synthesis when added to cell-free systems[10–12] or hypotonically permeabilised cells[13], and the induction of (2′–5′) oligo(A) synthetase was correlated with the antiviral action of interferon[14,15]. In the present work, the involvement of this enzyme in the antigrowth effect of interferon was studied. We show that addition of the (2′–5′) oligo(A)nucleotides to the culture medium of intact lymphocytes mimics the antimitogenic effect of interferon. Experiments with lymphocyte extracts show that, while interferon increases the (2′–5′)oligo(A) synthetase E, the mitogenic stimulus of concanavalin A leads to a decrease in (2′–5′) oligo(A) accumulation.

Table 1 Inhibition of DNA synthesis in Con A-stimulated mouse spleen lymphocytes by interferon and by (2′–5′) oligo-isoadenylate

Addition to Con A-stimulated cultures	Time of addition	DNA synthesis at 72 h ^{3}H-thymidine incorporated, c.p.m.	Inhibition (%)
Experiment 1			
None	—	111,020	0
Interferon, 200 U ml^{-1}	0 h	13,175	88
Interferon, 200 U ml^{-1}	24 h	66,050	41
Interferon, 200 U ml^{-1}	48 h	102,060	8
Experiment 2			
None	—	66,150	0
(2′–5′)ApApApA, 5 μM	0 h	17,665	74
(2′–5′)ApApApA, 5 μM	24 h	18,800	72
(2′–5′)ApApApA, 5 μM	48 h	56,765	15

Mouse spleen lymphocytes (8×10^6 cells per ml) were treated with 2.5 μg ml^{-1} Con A. Interferon (200 U ml^{-1}) or (2′–5′)ApApApA (5 μM) were added 10 min later (zero time) or at various times after Con A. DNA synthesis was measured at 72 h by a 3-h pulse of 6 μCi ml^{-1} ^{3}H-thymidine (5 Ci mmol^{-1}). Cells were then collected on glass fibre filters (Whatman GFC), washed twice with 5 ml cold PBS, twice with 5 ml 5% TCA then with 95% ethanol, dried and counted. ^{3}H-thymidine incorporated into the unstimulated lymphocytes (28,750 c.p.m.) was subtracted from that incorporated into the mitogen-treated cells.

The mitogenic stimulation of mouse spleen lymphocytes by concanavalin A (Con A) was studied. Spleens of BALB/c mice (8–10 weeks old) were teased through a metal mesh and cells were suspended in Dulbecco's phosphate-buffered saline (PBS). Erythrocytes were lysed in 0.01 M $KHCO_3$, 0.155 M NH_4Cl and 0.1 mM EDTA, and cells were washed twice with PBS. Lymphocytes were cultured at $4–8 \times 10^6$ cells per ml in RPMI 1640 medium (Gibco), or when indicated in DMEM, supplemented with 5% heat-inactivated fetal calf serum, penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). Cultures of 1 ml were incubated at 37 °C, in 5% CO_2–95% air (in 17 × 100 mm plastic tubes, Falcon No. 2051). Con A (Miles Yeda) was used at 2.5 μg ml^{-1} which gave optimal stimulation of ^{3}H-thymidine incorporation. Mouse L cell interferon was prepared and purified partially to 10^7 U per mg protein, as previously described[16].

In Con A-stimulated lymphocytes, DNA synthesis started at 48 h and was maximal by 72 h. Interferon added immediately after Con A, strongly reduced DNA synthesis measured at 72 h. The dose of interferon that caused 50% inhibition of thymidine incorporation ranged from 130 to 250 units ml^{-1} in several experiments. The inhibitory effect of interferon on subsequent DNA synthesis gradually decreased if interferon was added after the cells had been exposed to Con A (Table 1). In this experiment, when interferon (200 U ml^{-1}) was added by 24 h, the inhibition was only half of that measured at zero time, and by 48 h the inhibition was very small.

The activity of the (2′–5′) oligo(A) synthetase E was studied in stimulated lymphocytes, in conditions where interferon strongly inhibits subsequent DNA synthesis. Cytoplasmic extracts were prepared with NP40 and (2′–5′) oligo(A)synthetase measured after binding to poly(rI)·(rC) agarose. Table 2 shows that 24 h after adding interferon to unstimulated resting lymphocytes, as well as to Con A-stimulated cells, the activity of the (2′–5′) oligo(A)synthetase is increased. The basal activity without interferon was higher in these cells than that in L cells[14], but there was no difference in the basal level of unstimulated and mitogen-stimulated lymphocytes. The double-stranded RNA-dependent eIF-2 kinase Pk-i[12] was also clearly induced in the interferon-treated lymphocytes (not shown).

As interferon induces an increase in (2′–5′) oligo(A) synthetase E in the lymphocytes, we determined whether exogenously added (2′–5′) oligo(A) could mimic the inhibitory effect of interferon on DNA synthesis. Because highly charged

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Table 2 (2′–5′) Oligo-isodenylate synthetase E activity in mouse spleen lymphocytes

	Poly (rI):(rC) bound fraction from	Oligo-isoadenylate synthesis [^{32}P]-(2′–5′)ApA (c.p.m.)
Expt 1	Untreated cells	3,315
	+interferon 200 U ml^{-1}	7,785
	+ interferon 100 U ml^{-1}	12,117
Expt 2	Untreated cells	1,225
	+Con A 2.5 g	1,305
	+Con A +interferon 1000 U ml^{-1}	3,910
	+interferon, 1000 U ml^{-1}	2,590

Lymphocyte cultures (4–8 × 10^6 cells per ml) in DMEM were treated for 24 h as indicated. 4 × 10^7 cells (expt 1) or 2.4 × 10^7 (expt 2) were washed twice with 35 mM HEPES buffer *p*H 7.5, 140 mM KCl, 3 mM $MgCl_2$ and lysed in 0.1 ml of 20 mM HEPES buffer *p*H 7.5, 120 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol (buffer B) containing 0.5% Nonidet P40 (NP40). Lysates were centrifuged for 6 min in an Eppendorf centrifuge (8,000*g*) and 20–30 μl supernatant (expt 1, 16 μg protein; expt 2, 8 μg) mixed with 25 μl packed poly(rI)·(rC)-Agarose beads (PL Biochemicals) for 5 min at room temperature. The non-adsorbed material was removed by centrifugation, the pellet washed with buffer B and 10 μl of 2.5 mM [^{32}P]ATP (3 Ci mmol^{-1}) and 2.5 mM dithiothreitol were added. The suspension was incubated 20 h at 30 °C and 6 μl of the supernatant was treated 30 min at 37 °C with 0.2 units of BAP in 30 mM Tris base. 3 μl were applied to thin layer sheets of polyethylenemine (Schleicher and Schull) and chromatographed in 0.5 M acetic acid. Enzyme E activity was determined as before[12] by counting the ^{32}P-(2′–5′)ApA formed.

nucleotides would not penetrate intact cells, we tried the (2′–5′) oligo(A) without 5′ phosphates, that is the bacterial alkaline phosphatase (BAP) resistant nucleotide cores. A series of oligomers (chemically synthesised by Rapoport and Lapidot[17]) were added to the medium of Con A-stimulated lymphocytes. Table 3 shows that (2′–5′)ApApA or longer oligomers (tetramer and pentamer) efficiently inhibit the Con A-stimulated DNA synthesis. Concentrations of 2–5 × 10^{-6} M were effective but below 1 μM the trimer had little effect on DNA synthesis in these intact lymphocytes. The effect is specific since the (3′–5′)ApApA is devoid of inhibitory activity (Table 3). Williams and Kerr[13] showed that the BAP resistant cores of (2′–5′)pppApApA were able to inhibit ^{35}S-methionine incorporation in permeabilised cells, whereas in cell-free systems of protein synthesis these BAP-treated nucleotides were inactive[10]. Possibly, the intact cell can convert the cores into the active form by phosphorylation, a function which is lost in cell-free systems.

Adding (2′–5′)ApApApA to lymphocytes 24 h after Con A stimulation was as effective as at time zero, whereas at 48 h or later the oligonucleotide did not inhibit subsequent DNA synthesis (Table 1). This suggests that (2′–5′) oligo(A) inhibits some events preceding entry into S phase.

The above experiments suggest that the increase in (2′–5′) oligo(A) synthetase E produced by interferon treatment, could mediate the inhibition of DNA synthesis in stimulated lymphocytes. When (2′–5′) oligo(A) synthetase E was measured after isolating the enzyme from the cell extract with poly(rI)·(rC)-Agarose, Con A treatment neither reduced the basal level of the enzyme in lymphocytes nor its induction by interferon (Table 2). However, Table 4 shows that extracts of Con A-stimulated lymphocytes contain an activity that inhibits the synthesis of (2′–5′) oligo(A) if added to a known amount of enzyme E. Thus, the amount of (2′–5′) oligo(A) formed by the enzyme was much smaller in the presence of extracts from Con A-stimulated lymphocytes than with those from lymphocytes maintained without mitogen. Fresh splenic lymphocytes (time zero in Table 4) also contain the inhibitor. The level of inhibitor of (2′–5′) oligo(A) synthesis is, therefore, high in lymphocytes in which DNA synthesis is active: fresh splenic lymphocytes synthesise DNA and this decreases rapidly unless a mitogen is added to the culture[18]. Similarly, the level of inhibitor of (2′–5′) oligo(A) formation decreases when the lymphocytes are maintained in culture without mitogen, but increases if Con A is added. The differential inhibition of (2′–5′) oligo(A) synthesis was maximum at 72 h after Con A stimulation, but was already marked at 48 h and even 24 h after initiating the cultures (Table 4). The nature of the inhibitor is unclear: it is heat labile and non-dialisable (not shown). Preliminary results show that extracts of Con A-stimulated lymphocytes contain an activity which degrades the oligonucleotide formed, as does the 2′-phosphodiesterase described previously[19,20].

The Con A mitogenic stimulus is, therefore, accompanied by a high level of an inhibitor of (2′–5′) oligo(A) synthesis, which could lower the basal rate of oligonucleotide formation and allow entry into S phase. This is supported by the fact that exogenous (2′–5′) oligo(A) addition inhibits entry into S phase. The effect of interferon on Con A-stimulated lymphocytes may be explained by the induction of the (2′–5′) oligo(A) synthetase E to a level which exceeds the activity of the inhibitor (the latter remained unchanged after interferon; not shown). Since cellular

Table 3 Effect of different oligonucleotides on DNA synthesis in Con A-stimulated mouse spleen lymphocytes

Oligo(A) added	Final concentration (μM)	Inhibition of incorporation of ^{3}H-thymidine (%)
None	—	0
(2′–5′) trimer	2	81
A2′p5′A2p5′A	5	88
	10	87
(3′–5′) trimer	5	0
A3′p5′A3′p5′A	10	0
(2′–5′) tetramer	1	0
A2′p5′A2′p5′A2′p5′A	5	50
	10	73
(2′–5′) pentamer	5	64
A2′p5′A2′p5′A2′p5′A2′p5′A	10	94

Mouse spleen lymphocytes (8 × 10^6 cells per ml) were treated with 2.5 μg ml^{-1} Con A and 10 min later the different oligonucleotides (chemically synthesised as in ref. 17) were added. DNA synthesis was measured at 72 h as described in Table 1. The total ^{3}H-thymidine incorporated into TCA insoluble material of 1 ml suspension in the absence of any oligonucleotide was 125,000 c.p.m. for the stimulated lymphocytes. Per cent inhibition was calculated after the values of unstimulated cells (32,800 c.p.m.) was subtracted.

Table 4 Inhibition of the (2′–5′) oligo-isoadenylate synthetase in Con A-stimulated mouse spleen lymphocytes

Lymphocyte extracts prepared at:	Inhibition of (2′–5′) oligo(A) synthesis	
	No mitogen (%)	Con A (%)
0 h	60*	—
24 h	45	60
48 h	15	60
72 h	15	80

Extracts (0.1 ml) from 5 × 10^7 Con A-stimulated lymphocytes or 10^8 untreated cells were prepared at 0, 24, 48 and 72 h as in Table 2. Cell viability in the unstimulated culture was high (>90%) throughout the experiment as determined by staining with Trypan blue. The inhibitor of (2′–5′) oligo(A) accumulation was measured as follows: Insoluble (2′–5′) oligo(A) synthetase was prepared by mixing 165 μg protein from the 0.5 M KCl ribosome extract from interferon-treated L cells[12] with 0.3 ml packed poly(rI):(rC)-Agarose as in Table 2. To 20 μl packed beads, 6 μl of 1.5 mM [α-^{32}P]ATP (3 Ci mmol^{-1}) and 10 μl of various concentrations of lymphocyte extracts (5–15 μg) were added. After 2 h at 30 °C the synthesis of (2′–5′) oligo(A) was measured as in Table 2. The inhibition was calculated for 8 μg protein extract. Without extract 2,570 c.p.m. of ^{32}P-(2′–5′)ApA was formed.

* Fresh splenic lymphocytes before culture.

RNAs activate enzyme E[17], interferon could lead to accumulation of the oligonucleotides and, as found for exogenously added (2′–5′) oligo(A), inhibit entry into S phase. It will be interesting to establish whether the inhibitor of (2′–5′) oligo(A) synthesis, described here, exists in other cells and is increased in fast growing cells. It will also be important to determine whether the antimitogenic effect of exogenous (2′–5′)ApApA results from phosphorylation of the oligonucleotide in the cell and activation of RNase F[20,21] or from some other function of these oligonucleotides. Finally, the (2′–5′) oligo(A) may become useful to regulate the immune response. [Recently, E. Martin (personal communication) reported that the (2′–5′)ApApA also inhibits DNA synthesis in cultures of lymphoblastoid Daudi cells.]

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Ionomycin stimulates mast cell histamine secretion by forming a lipid-soluble calcium complex

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The activation of cellular function following the direct introduction of Ca^{2+} into the cytosol by the use of a Ca^{2+}-transporting ionophore has served to confirm the widely held idea that Ca^{2+} has the status of a second messenger in many cell types[1,2]. However, this evidence has been obtained largely from the use of a single ionophore, the antibiotic A23187; experiments with X537A, which is another ionophorous antibiotic capable of transporting Ca^{2+} (ref. 3), have failed to show the expected characteristics. For example, histamine release from rat mast cells mediated by X537A is neither dependent on extracellular Ca^{2+} nor prevented by metabolic inhibitors[4]. Ionomycin is a recently described polyether antibiotic produced by *Streptomyces conglobatus* ATCC 31005, and is active against Gram-positive bacteria[5]. The antibiotic action is presumably due to its ionophorous properties, as it extracts Ca^{2+} from an aqueous phase into an organic phase with a stoichiometry of 1:1 (ref. 6). The ionophore is also capable of transporting $^{45}Ca^{2+}$ across biological membranes (our unpublished results). Here we report the application of ionomycin to rat mast cells. We show that ionomycin stimulates mast cell secretion solely through its ability to form a lipid-soluble calcium complex, and thus to convey Ca^{2+} across the hydrocarbon region of the cell membrane.

Ionomycin-mediated histamine secretion is completely dependent on the presence of extracellular Ca^{2+}, although, as shown in Fig. 1, the actual concentration of Ca^{2+} required varies inversely with the concentration of the ionophore used. The secretion seems to occur by the normal exocytotic mechanism, as the cells show no loss of the cytoplasmic marker enzyme, lactate dehydrogenase (<5% at 5 μM ionomycin), and secretion is prevented when cell metabolism is inhibited by preincubation for 10 min with antimycin (5 μM) in the absence of glucose.

The partial secretions induced at suboptimal concentrations of Ca^{2+} in the curves of Fig. 1 represent completed secretory events; they are not the consequence of reduced rates of exocytosis. Secretion at different suboptimal ionophore concentrations occurs at similar rates with $t_{1/2} \sim 0.6$ min and completion by 6 min (Fig. 2). At higher ionomycin concentrations (2.5 μM) secretion stops abruptly when the histamine which is available for secretion (normally about 80% of total) is exhausted.

There was little further secretion on treatment with additional ionomycin after secretion due to a first suboptimal dose was complete; this suggests that the characteristic time course for cessation of secretion reflects termination by some cellular mechanism. As shown in Fig. 2, application of 1.5 μM ionomycin in two stages (each of 0.75 μM) caused rather less secretion than 1.0 μM ionomycin at a single application. Such characteristics might result from a decrease in the sensitivity to internalised Ca^{2+} following its initial elevation due to the ionophore (for example, by the recruitment of additional Ca^{2+}-pumping or Ca^{2+}-buffering capacity) or by a termination mechanism operating at a subsequent stage in the Ca^{2+}-initiated sequence of events which lead to secretion.

Of the Ca^{2+} present in the extracellular medium, only that fraction which is present as a calcium–ionomycin complex will be able to partition into the cell membrane and thence into the cytoplasm. To estimate the size of this fraction, we used an organic extraction technique[7,8] to measure the equilibrium

$$H_2I_{org} + Ca^{2+}_{aq} \rightleftharpoons Ca.I_{org} + 2H^{+}_{aq} \quad (1)$$

H_2I and I^{2-} represent the protonated and fully dissociated forms of ionomycin, respectively. The dependence on the aqueous Ca^{2+} concentration of the amount of $^{45}Ca^{2+}$ extracted into an organic phase is shown in Fig. 3.

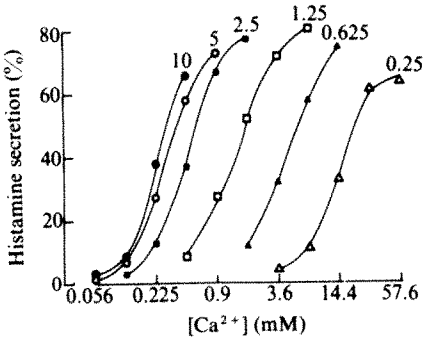


Fig. 1 The concentration dependence on Ca^{2+} of histamine secretion due to different concentrations of ionomycin. Rat peritoneal mast cells were isolated and purified as previously described[15] and suspended at $\sim 10^5$ cells per ml in a buffered salt solution, *p*H 7.5, comprising 137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 10 μM EGTA, 20 mM HEPES, 5.6 mM glucose and 1.0 mg ml^{-1} bovine serum albumin. Solutions were equilibrated to 37 °C before addition of 0.2 ml cells to tubes containing ionomycin and $CaCl_2$ to give the indicated concentrations in a final volume of 0.5 ml. Secretion was terminated after 10 min by the addition of 1.0 ml ice-cold phosphate-buffered saline and the cells were centrifuged at 4 °C. The histamine content in the supernatants was determined fluorimetrically[15] and is expressed as a percentage of total cell histamine. Data points represent the means of duplicate determinations which were within 10% of each other. Concentrations of ionomycin (μM) are indicated above each concentration–effect curve.

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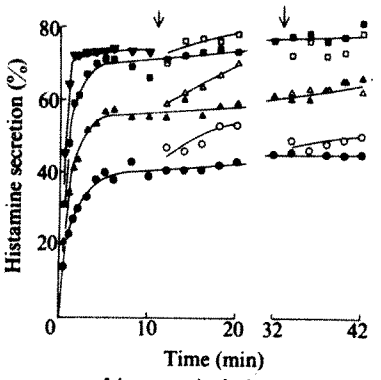


Fig. 2 Time courses of ionomycin-induced secretion. Secretion was initiated by adding 5 ml cells ($\sim 1.5 \times 10^5$ cells per ml) to buffer containing $CaCl_2$ (final concentration 1.8 mM) and ionomycin, giving a total volume of 20 ml. At the times indicated by vertical arrows, 3 ml was transferred to another tube containing additional ionomycin (in less than 10 μl) sufficient to double the concentration. Samples (0.5 ml) from these incubations were quenched at different times by addition to 1.0 ml ice-cold phosphate-buffered saline and the extent of histamine secretion was determined as in Fig. 1. Curves show time courses of secretion due to ionomycin at: ●, 0.75 μM; ▲, 1.0 μM; ■, 1.25 μM; ▼, 2.5 μM. Additional secretion due to doubling of ionomycin after the initial secretion was complete is shown: ○, 0.75–1.5 μM; △, 1.0–2.0 μM; □, 1.25–2.5 μM.

The solubility of inorganic ions in low dielectric organic phases is extremely small (ref. 1, p. 45), so the initial stage in the complexation by ionomycin is likely to occur in the aqueous phase; this can be concluded from kinetic studies[9] and from the use of fluorescent probe techniques[10] in the analogous reaction of alkali-metal ionophores. As evidence for the existence of the complex in the aqueous phase, we find a Ca^{2+}-dependent change in the UV spectrum of aqueous ionomycin, with an absorption maximum at 305 nm for the difference spectrum. Equation (1) can therefore be considered as the sum of four component equilibria

$$H_2I_{org} \rightleftharpoons H_2I_{aq} \quad (2)$$

$$H_2I_{aq} \rightleftharpoons HI^-_{aq} + H^+_{aq} \rightleftharpoons I^{2-}_{aq} + 2H^+_{aq} \quad (3)$$

$$I^{2-}_{aq} + Ca^{2+}_{aq} \rightleftharpoons Ca.I_{aq} \quad (4)$$

$$Ca.I_{aq} \rightleftharpoons Ca.I_{org} \quad (5)$$

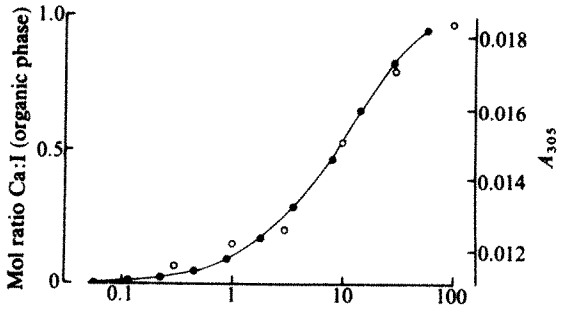


Fig. 3 Extraction of Ca^{2+} into organic phase by ionomycin. The transfer of radioactivity from an aqueous phase (the buffered salt solution described in Fig. 1 legend) containing different concentrations of $^{45}CaCl_2$ ($\sim 3 \times 10^6$ c.p.m. μmol^{-1}) into an equal volume of organic phase (toluene/butanol, 7:3) containing 20 μM ionomycin was measured as previously described[8]. The extraction of Ca^{2+} is expressed as a mol fraction of the ionomycin present (solid symbols). The figure also shows the dependence on calcium concentration of the spectral change of aqueous ionomycin. The absorbance at 305 nm was measured for ionomycin (1 μM) in 1 ml buffered salt solution (as described in Fig. 1 legend, but omitting $MgCl_2$, which also caused a spectral change, and albumin, which absorbs at this wavelength) containing different concentrations of $CaCl_2$. Higher concentrations of ionomycin could not be used because at the highest calcium concentrations visible precipitation occurred in the cuvette.

Equations (2) and (5) represent the partition coefficients of ionomycin and its calcium complex; the aqueous equilibrium is described by equations (3) and (4), with equation (3) representing the acid dissociation of ionomycin, which remains constant throughout our experiments (because we work at constant pH). The two experiments described by Fig. 3 show that the extent to which ionomycin extracts Ca^{2+} into an organic phase (closed symbols, equation (1)) is indicative of the aqueous equilibrium between the free acid and Ca^{2+} (open symbols, equation (4)), as both reactions show a similar dependence on aqueous phase Ca^{2+}.

In the buffered salt solution used in our experiments, ionomycin has a rather low affinity for Ca^{2+} (Fig. 3). Ionomycin has $pK_a \sim 8.3$ (ref. 6) so that in our buffered salt solution ($pH = 7.5$), protonation of ionomycin (equation (3)) occurs in preference to the formation of the calcium complex (equation (4)). The affinity for Ca^{2+} is very much higher at pH 10 (ref. 6). Using the data of Fig. 3 we have estimated the aqueous phase calcium–ionomycin concentration for the data points of Fig. 1 (and for another similar experiment) and the histamine secretion is plotted in Fig. 4 as a function of calcium–ionomycin.

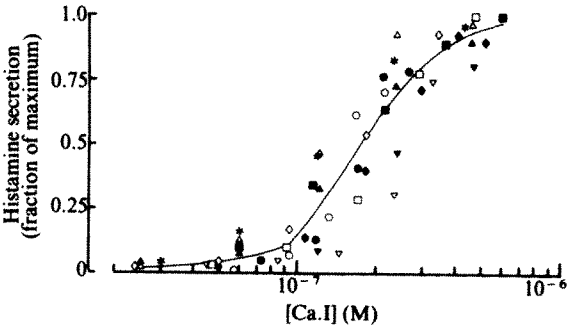


Fig. 4 The dependence of histamine secretion on the calculated concentration of calcium–ionomycin complex. The fraction of ionomycin which was present as the calcium–complex at a given $CaCl_2$ concentration (Fig. 3) was used to determine the calcium–ionomycin concentration for the data points of Fig. 1 (solid symbols) and another similar experiment (open symbols). For each experiment histamine secretion was normalised as a fraction of the maximum secretion observed. Total ionomycin concentrations were: ▼, 10 μM; ▲, △, 5 μM; *, 2.5 μM; ◇, 2 μM; ■, 1.25 μM; □, 1.0 μM; ◆, 0.625 μM; ▽, 0.5 μM; ●, 0.25 μM; ○, 0.2 μM.

The assumption that ionomycin-induced histamine secretion can be attributed to the aqueous concentration of calcium–ionomycin is supported by the data in Fig. 4. Concentration–effect curves falling over a 70-fold range of Ca^{2+} concentrations (Fig. 1) are encompassed within a twofold scatter about a curve which indicates cell stimulation over a calcium–ionomycin range of 0.1–0.3 μM. Although calcium–ionomycin has access to the cell interior, these concentrations do not represent the range over which cytoplasmic Ca^{2+} will be elevated. At the low concentrations of ionised Ca^{2+} believed to exist in the cytoplasm[11], calcium–ionomycin will rapidly and completely dissociate as soon as it enters the cell, and it is the rate of this partition process which will be a function of the external aqueous calcium–ionomycin concentration.

Our results indicate that it is the rate of entry of calcium into the cell which controls the extent (and not kinetics) of secretion. For normal (receptor-mediated) secretion, this implies that the extent of secretion will depend on the number of Ca^{2+} channels, and thus, presumably, on the number of stimulated receptors. The consequent intracellular signal could be an elevated concentration of cytoplasmic Ca^{2+}, occurring as a result of the relative kinetics of the ionophore-mediated Ca^{2+} influx and cellular Ca^{2+}-buffering mechanisms; this is likely to be of the order of 1 μM as found with other systems[12,13]. Alternatively, as Ca^{2+} influx occurs at the plasma membrane and Ca^{2+} sequestration occurs elsewhere (mitochondria, secretory granules), it could be that it is the Ca^{2+}-concentration gradient necessarily set up by the opposing effects of these two processes which acts

as the second messenger in exocytosis. A triggering role for a Ca^{2+} gradient might explain the directionality of exocytosis due to a localised stimulus[14].

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Acetylcholine synthesis by mesencephalic neural crest cells in the process of migration *in vivo*

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Specific to the vertebrate embryo, the neural crest is a transitory structure whose constituent cells migrate extensively through the developing animal and ultimately give rise to many distinct cell types, including the components of the peripheral nervous system[1,2]. The earliest clear indices of their differentiation have so far been detected only when cells from the crest have reached their destination. This is exemplified by the acquisition of the ability to synthesise and store catecholamines; absent from crest cells before and during their dorso–ventral migration, this ability appears concomitantly with their aggregation into the primary sympathetic ganglia[3–6]. The chronology of cholinergic maturation, however, is less well defined. Appropriate biochemical markers are demonstrable as soon as parasympathetic or enteric ganglia are formed[7–9], but the lack of a suitable cytochemical method is a major obstacle to the identification of any cholinergic cells before then. Although acetylcholinesterase (AChE) is present in migrating neural crest[10], choline acetyltransferase (CAT), the enzyme catalysing acetylcholine (ACh) synthesis, is a much more relevant correlate[7,11,12], and definitive evidence for cholinergic differentiation should include the demonstration of ACh-synthesising activity in intact cells[13,14] or their extracts. We show here that neural crest, as soon as it begins migration, can synthesise ACh.

To overcome the difficulty of applying biochemical techniques to neural crest cells soon after they have begun to migrate, we took advantage of the fact that mesencephalic neural crest in Japanese quail embryos of 8–12 somites is clearly visible underneath the ectoderm as a sheet of cells on either side of the mesencephalic vesicle, and can be dissected out mechanically[15]. Each discrete fragment of tissue so obtained is made up of about 1,500 cells and is free from contamination by neural tube or mesenchyme (Fig. 1*b*). This system provides a unique opportunity for the preparation and study of a homogeneous population of cells actually migrating *in vivo*. Furthermore, it is equally possible to remove the mesencephalic crest at a slightly earlier stage, when it is still in the form of neural folds and migration is just beginning (Fig. 1*a*). Each crest fragment at this time contains approximately 1,000 cells.

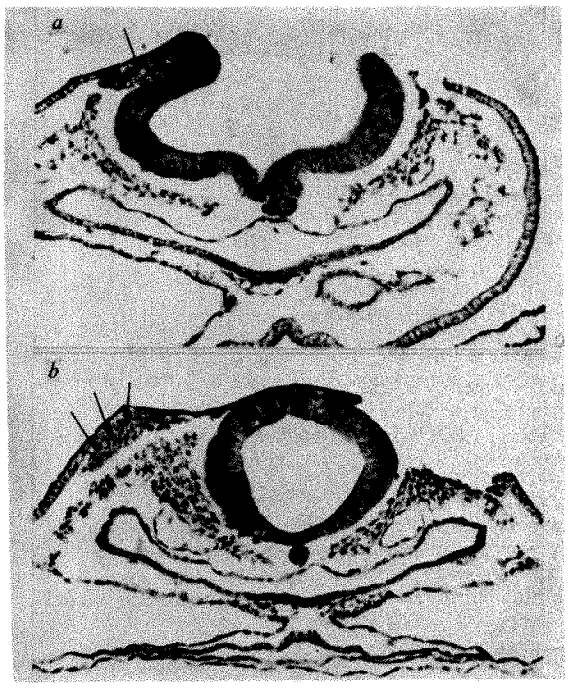


Fig. 1 Removal of the mesencephalic neural crest at the onset of, and during, the migration process in early quail embryos. *a*, Transverse section at the mesencephalic level of a quail embryo of 7 somites stained with haematoxylin. On one side, the migration of crest cells from the neural fold (arrow) is starting. At the opposite side, the crest has been cut out, together with the ectoderm, by means of a microscalpel (×110). *b*, Transverse section of a 9-somite quail embryo, stained with haematoxylin, showing the mesencephalic neural crest as a thin multi-layered sheet of cells immediately below the ectoderm. Migration is well under way. (Orthotopic grafts of quail neural tube into chick embryos[2] before the onset of crest migration have shown that the group of cells designated by the arrows on the intact side is made up of a pure population of crest cells.) Again, the contralateral crest has been excised. Note the complete and exclusive removal of the crest cells and the absence of damage to the neural tube (×110). (In this report, crest obtained from embryos of 5–7 somites is referred to as 'neural fold', that from embryos of 8–12 somites as 'migrating crest'.)

On incubation with ^{3}H-choline, isolated migrating mesencephalic neural crest formed a labelled product that co-migrated with ACh on electrophoresis (Fig. 2*b*). The identity of the radioactive material was confirmed by the demonstration that 85–90% disappeared after AChE treatment (Fig. 2*c*, *d*). Crest isolated at the neural fold stage was also able to synthesise ACh, although in significantly smaller amounts (Fig. 2*a*). Mean values (fmol per 10^4 cells) obtained for ACh synthesis and accumulation during a 4-h incubation were 7.5 ± 2 ($n = 3$) and 25 ± 5 ($n = 5$) for neural fold and migrating crest, respectively; thus, the synthetic ability is about three times greater when the crest has left the neural primordium and is fully engaged in the migration process.

ACh synthesis implies the existence of CAT, and weak but measurable CAT activity was indeed found in homogenates of migrating neural crest (Table 1). However, unequivocal determination of very low CAT levels is difficult if carnitine acetyltransferase (CarnAT) is present. This enzyme is, in fact, quite active in neural crest (Table 1, expts 3, 4). Although acetylation of choline by CarnAT is relatively inefficient[16,17], in some circumstances it may contribute significantly to ACh formation *in vitro*[18,19]. The use of CAT-specific inhibitors[20] can reveal such

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Table 1 Choline acetyltransferase and carnitine acetyltransferase activities in neural crest homogenates

Expt no.	Enzymatic activity (pmol product per min per mg protein) CAT	CarnAT	% Inhibition of ACh synthesis by NVP
1	3.6	ND	45
2	5.3	ND	57
3	2.2	28.1	60
4	2.8	31.2	52

In each experiment, 68–72 migrating crests (~10^5 cells, or 15 μg protein) were ultrasonicated in 10–20 μl 50 mM sodium phosphate, *p*H 7.4, and Triton X-100 was added to a final concentration of 0.5%. For CAT assay, the reaction mixture (total volume 20 μl) comprised: 5 μl homogenate, 12.5 mM choline chloride, 300 mM NaCl, 50 mM NaF, 0.1 mM eserine salicylate, 0.1 mM [1-^{14}C]acetyl CoA (Amersham; specific activity 58 mCi mmol^{-1}) and 50 mM sodium phosphate, *p*H 7.4. Each determination was made in the presence and absence of 0.5 mM NVP (Calbiochem). Incubations were carried out in the dark at 37 °C for 30 min and the reaction was stopped by adding 5 μl *p*H 1.9 electrophoresis buffer (5 times concentrated) containing 0.1 mg unlabelled ACh chloride. ACh was separated from unconverted acetyl CoA by high-voltage electrophoresis of the whole sample (5 kV, 40 min). Radioactivity in the ACh region of the paper was measured as described in Fig. 2 legend. Assay blanks, without enzyme, were run in an identical manner. The electrophoresis step, in addition to permitting the identification of the labelled reaction product, has the advantage of giving low blanks (45–65 c.p.m. including background, corresponding to ~0.03% the initial amount of ^{14}C-acetyl CoA); each experimental value was 1.5–3 times higher than the corresponding assay blank. For CarnAT determination, 12.5 mM DL-carnitine replaced choline in the reaction mixture, nonradioactive acetylcarnitine was added at the end of the incubation and the electrophoresis time was increased to 1 h. Results have been corrected for blanks, recovery and counting efficiency. Protein was estimated by the Lowry method, using bovine serum albumin as standard. ND, Not determined.

an artefact and the results obtained with one of them, 4-(1-naphthylvinyl)pyridine (NVP), are included in Table 1. At a concentration that does not affect CarnAT in quail tissue (unpublished results), NVP reduced ACh synthesis by an average of 54%. This is probably an underestimate, as NVP is not a particularly potent inhibitor[20,21].

Despite the inference that at least half the ACh produced in cell-free preparations was due to CAT, the involvement of this enzyme in ACh synthesis in whole cells remained to be assessed. Consequently, we examined the effect of saturating concentrations of carnitine on the conversion of ^{3}H-choline to ACh by intact neural crest cells. If CarnAT was responsible for the synthesis of significant amounts of ACh, labelling of the latter should be reduced in the presence of excess carnitine. Duplicate migrating mesencephalic neural crest preparations were incubated in medium containing both ^{3}H-choline and ^{3}H-carnitine (to monitor carnitine uptake and conversion to acetylcarnitine). In one case, a large excess of unlabelled carnitine was also present. The radioactivity accumulated by the cells in choline, carnitine and their acetylated products was subsequently determined in each sample. Table 2 shows the results of an experiment in which the concentration of unlabelled carnitine was more than 1,000 times that of the radioactive precursors. It can easily be calculated that this massive dose increased the intracellular carnitine concentration by a factor of about 500; at the same time, the total radioactivity in acetylcarnitine was reduced by over 75%. In marked contrast, neither the size of the choline pool nor the labelling of ACh was significantly affected. This result clearly shows that choline and carnitine do not compete for the same enzymatic sites in intact cells and strengthens our contention that CAT is responsible for ACh synthesis in neural crest.

We conclude from these studies that mesencephalic crest cells possess a fundamental marker of cholinergic metabolism from the onset of the migratory phase of their development. This may reflect very early differentiation of the fraction of the population that will later give rise to neurones of the ciliary ganglion[22,23]. On the other hand, ACh-synthesising activity may not necessarily be restricted to presumptive parasympathetic nerve cells. It has been proposed that expression of cholinergic traits is an intrinsic property of differentiating neuroblasts, irrespective of their

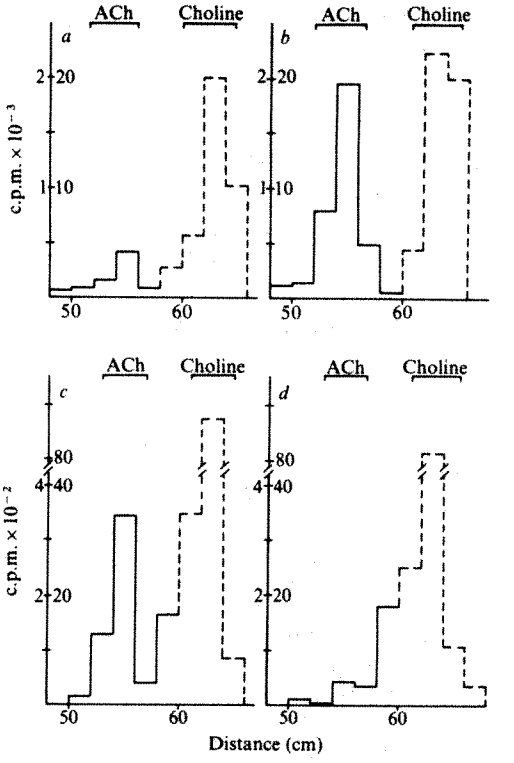


Fig. 2 ACh synthesis by excised mesencephalic crest. The technique was based on that described by Mains and Patterson[14]. The incubations were carried out in 200 μl of medium (L15 lacking choline) containing 15 μM [methyl-^{3}H]/choline (Amersham, 6.4 or 36 Ci mmol^{-1}) and 10^{-5} M eserine salicylate. After 4 h at 37 °C in humidified air, the medium was withdrawn, the tissue fragments were washed twice with cold Tyrode's solution and disrupted ultrasonically in 15 μl of electrophoresis buffer, *p*H 1.9 (ref. 13), containing unlabelled choline and ACh. The entire sample was applied to Whatman 3 MM paper and electrophoresed at 5 kV for 1.75 h (ref. 13). The positions of ACh and choline were detected with an iodine-based spray[25] and sequential, 2-cm-wide rectangles were cut out in the appropriate regions and counted in 10 ml of Beckman 'HP' scintillation fluid after elution/decolouration with 1 ml $Na_2S_2O_3$ (0.5% w/v in H_2O). Counting efficiency was 45–50%. Background values (35–50 c.p.m.) were obtained from the rectangles immediately preceding the ACh spot. The diagrams show the distribution of radioactivity migrating with ACh (solid lines, outer scale) and choline (broken lines, inner scale), the positions of the marker spots and the total distance of migration towards the cathode. *a*, *b*, Comparison of ACh synthesis by samples consisting of 20 neural folds (*a*) and 20 migrating crests (*b*). The specific activity of the ^{3}H-choline was 36 Ci mmol^{-1}. The radioactive ACh peak in *b* was not notably altered by prior purification[14] of the precursor, nor by removal of the ectoderm layer associated with the crest. *c*, *d*. Effects of AChE. The sample consisted of 50 migrating crests. After incorporation of ^{3}H-choline (6.4 Ci mmol^{-1}), the cells were ultrasonicated at *p*H 4 and centrifuged. The supernatant was adjusted to *p*H 7 and divided into two equal parts. Each was incubated for 15 min at 25 °C in the absence (*c*) and presence (*d*) of purified *Electrophorus electricus* AChE (Sigma), followed by acidification with concentrated *p*H 1.9 buffer and electrophoresis. Background counts have been subtracted.

Table 2 Effect of excess carnitine on ACh synthesis

Concentration of unlabelled DL-carnitine	Total net radioactivity (c.p.m.) recovered as: Carnitine	Acetyl-carnitine	Choline	Acetyl-choline
0	1,025	280	8,900	380
40 mM	400	65	8,300	400

Each sample consisted of 20 migrating crests (3×10^4 cells). The first was incubated in medium (L15 without choline) containing 10^{-5} M eserine salicylate, 100 μCi ml^{-1} (15 μM) [Me-^{3}H]choline and 52 μCi ml^{-1} (30 μM) DL[Me-^{3}H]carnitine (Amersham). The second sample was incubated in medium containing identical amounts of the radioactive compounds, but to which unlabelled DL-carnitine had been added to a final concentration of 40 mM, thus reducing the specific activity of the precursor from 1.7 Ci mmol^{-1} to 1.3 mCi mmol^{-1}. (Preliminary experiments had shown that extracellular carnitine concentrations of this order were necessary to increase the intracellular level sufficiently to saturate CarnAT.) After incubation for 4 h, the samples were treated as described in Fig. 2 legend, except that unlabelled carnitine and acetylcarnitine markers were added before ultrasonication and the electrophoresis time was increased to 2.25 h. In these conditions, labelled precursors and acetylated products were well separated from one another. The total radioactivity in the regions of the paper corresponding to the substances of interest was determined as indicated in Fig. 2 legend. Background values have been subtracted.

ultimate fate[24]; it might even be a feature of all neural crest cells (possibly related to the migration process[10]). This uncertainty could be resolved directly only by the development of an appropriate, unambiguous cytochemical test. In the meantime, a useful first step towards elucidating the relationship between the early cholinergic phenotype and ontogeny of the peripheral nervous system would be to determine whether crest cells from other axial levels also synthesise ACh and, if so, whether this ability is retained during at least the first stages of sympathetic and sensory ganglion formation.

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Dopamine inhibition of action potentials in a prolactin secreting cell line is modulated by oestrogen

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Secretory activity of the anterior pituitary gland is regulated by the brain through stimulatory and inhibitory substances released from nerve endings in the median eminence of the hypothalamus and carried by the adenohypophysial portal blood system to their respective target cells. These hypothalamic influences are modulated by the feedback action of peripheral hormones. Prolactin (PRL) secreting cells are, at least partially, under the stimulatory influence of thyrotropin releasing hormone (TRH)[1,2] and of oestrogens[3]. However, they are mainly controlled by inhibitory substances among which dopamine (DA) is one of the most potent *in vivo* as well as *in vitro*[4,5]. The inhibitory effect of DA is reversed by oestrogen *in vitro*[6]. The mechanism by which these factors interact on their target cells is poorly understood. The recent discovery that anterior pituitary cells are excitable and that they are able to generate Ca^{2+}-dependent action potentials has led to the suggestion that these effects are involved in a stimulus-secretion coupling at the membrane level[7–9]. In this paper, we report that DA inhibits both the spontaneous and TRH-induced action potentials in clonal PRL pituitary cells. In addition, oestradiol-17β is able to reverse the inhibitory effect of DA.

In this study GH3 B6 cells, a subclone of GH3 rat prolactin cell line were used. These cells secrete prolactin and respond to both TRH and oestradiol[2]. Stable intracellular recordings lasting between 10 min and 1 hour were obtained from 260 cells. Of the cells tested 51% were electrically excitable and displayed action potentials when depolarised by an outward current. Twenty-six per cent of the cells were spontaneously active. These action potentials were mainly Ca^{2+}-dependent (Fig. 1*a*).

Sixty-two spontaneously firing cells were tested for an effect of DA; in 25 cells (40%), DA injected close to the cell provoked an inhibition of firing within 30–60 s (Fig. 1*c*). This inhibitory effect was preceded by a rapid decrease of the input resistance with no detectable change in the resting membrane polarisation (Fig. 1*b*); in 37 cells DA had no effect. In addition, DA inhibited

Table 1 Effect of ^{3}H-DA on PRL release and ^{3}H-DA binding in GH3 B6 cells

	Control	^{3}H-DA	^{3}H-DA + DA
PRL release (ng per mg cell protein)	283 ± 18.2	193 ± 19.8*	205 ± 6.9*
^{3}H-DA bound (d.p.m. per mg cell protein)	—	2,380 ± 196	676 ± 48

200,000 cells per 2-ml dish were grown for 5 days as previously described[10]. Before the experiment the cells were rinsed with warm Ham F10 + HEPES 20 mM, *p*H 7.2 (F10-H). They were thereafter incubated in 1 ml of fresh F10-H with or without the addition of ^{3}H-DA (16 pmol ml, 6.6 Ci mmol^{-1}) and unlabelled DA (10 nmol ml^{-1}) for 30 min at 37 °C in 95%–5% air–CO_2 humidified incubator. The incubation was stopped on ice. The cells were collected for protein assay and liquid scintillation measurement of bound radioactivity[10]. The half life of DA in such conditions was 30 min as measured in parallel dishes by J. P. Tassin (U. 114, INSERM, Collège de France) using a specific enzymatic radioassay. The medium was collected for PRL radioimmunoassay which was expressed in nanogram equivalent of rat standard RP-1 from the NIAMDD kit. Each point is the mean of triplicate ±s.e.m.

* $P < 0.05$.

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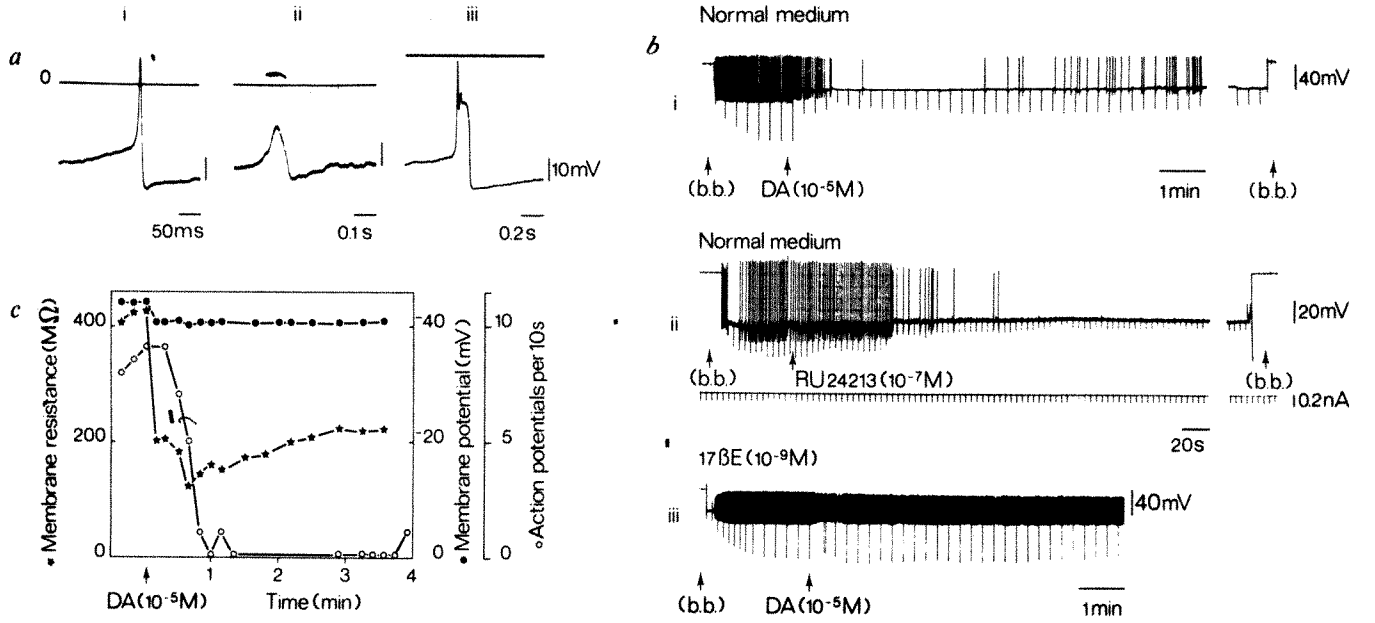


Fig. 1 Effect of DA on the firing of action potentials in the GH3 B6 prolactin secreting cell line. The cells were grown in Ham's F10 solution supplemented with 15% horse serum and 2.5% fetal calf serum. Cells were cultured for 5 to 7 days before being used in electrophysiological studies. The experiments were carried out in a temperature controlled room (26 ± 1 °C). Ten minutes before recording, the culture medium was replaced by a bathing solution with the following composition (in mM): NaCl 142.6; KCl 5.6; $CaCl_2$ 10; glucose 5; HEPES buffer 5 (*p*H 7.4). Stock solutions of oestradiol-17β(17β-E) were prepared in ethanol and diluted in the above solution; the final ethanol concentration being 0.01%; TRH (Beckmann) and DA (Hoffman-La Roche) were dissolved in bathing solution. The substances to be tested were delivered via a pneumatic ejection system which allowed rapid administration (0.5 s) of very small volume (1–5 nl) of solution from micropipettes positioned close to the membrane of the cell tested[13]. Twenty-six per cent of the cells ($n = 260$) were spontaneously active.

a, The all-or-none action potential had a positive overshoot and a prominent after-potential (i); the action potentials were reduced following the application of D 600, a known blocker of Ca^{2+} channels (ii); action potentials were still encountered in medium in which sodium chloride was replaced by choline chloride, however their size and shape were changed and no overshoot was observed (iii); the zero membrane potential is indicated by 0. *b*, The effect of DA on spontaneous action potentials. Vertical bars represent a measure of the membrane input resistance obtained by injecting a current of 0.16 nA with a bridge amplifier. i, Effect of DA on action potential firing and membrane resistance in an oestrogen depleted medium. ii, Effect of the DA agonist RU 24213 (Roussel-Uclaf) on action potential firing and membrane resistance in a oestrogen depleted medium. The current used to monitor the cell membrane resistance is shown at the bottom of the figure. In (i) and (ii) the bridge balance is indicated (b.b.). iii, Effect of DA when oestradiol-17β (10^{-9} M) was added to the recording medium. *c*, Time course of changes in membrane electrical properties following administration of DA (10^{-5} M) on the cell represented in b(i). ★, Membrane resistance; ○, frequency of firing of action potentials; ●, membrane potential.

the TRH-induced action potentials. The DA agonist RU24213 induced a response comparable to that of DA (Fig. 1*b*). In the same way as for DA, the inhibitory effect of RU24213 on action potential firing was always associated with a significant decrease in membrane resistance. Conversely, the DA antagonists haloperidol (10^{-6} M) and chlorpromazine (10^{-6} M) suppressed the inhibitory effect of DA on action potentials when they were administered to a spontaneously firing cell (Fig. 2*a*). Surprisingly, an increase in conductance occurred without any noticeable change in membrane potential. The spontaneously firing cells recorded in this study have a resting membrane potential of -45 ± 3 mV which is not near the normal equilibrium potential of any ions likely to be involved in the DA effect. The ionic nature of the currents involved in the DA effect has not yet been investigated. However, it is possible that DA increases the Cl permeability of the cell; a leakage of KCl from the tip of the KCl electrode may elevate the internal Cl concentration and bring the equilibrium potential for this ion close to −45 mV. Several arguments support this hypothesis: for example, when the resting membrane potential was displaced towards −80 mV, it was difficult to maintain a steady potential, although DA was able to induce a long-lasting depolarisation together with an increase in membrane conductance.

The DA inhibition of both spontaneous and TRH-induced action potentials may be related to the effect of DA on PRL secretion. A significant decrease of PRL release in GH3 B6 cells was induced within 30 min of exposure to ^{3}H-labelled DA (16 nM) (Table 1). Simultaneously the cells showed a binding capacity for ^{3}H-DA and this was partially inhibited by an excess of unlabelled DA (Table 1). The inhibitory effect of DA on action potential firing and the decrease in PRL secretion following exposure to DA are consistent with the stimulus–secretion coupling hypothesis. However, these two effects cannot be compared in a quantitative manner, as the method of DA application in the two experiments is different. Also, we do not know the exact amount of DA which reaches the cell membrane when DA is administered by pressure injection. We have previously shown that the DA agonist CB 154 decreased TRH-induced PRL release in GH3 B6 cells without modifying the binding of ^{3}H-TRH (ref. 10). Although these cells are of neoplasmic origin, they exhibit a binding capacity and a response to DA similar to that of normal anterior pituitary cells[5]. The fact

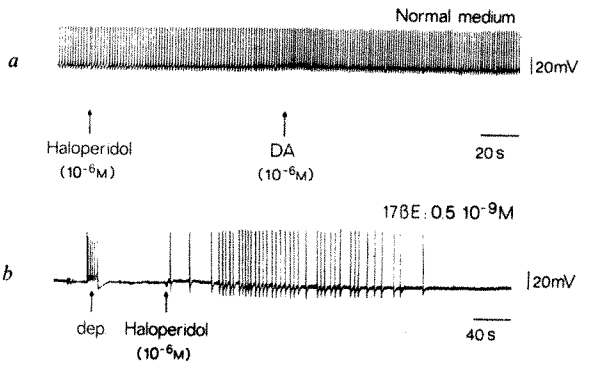


Fig. 2 Effect of haloperidol on action potential firing in GH3 B6 cells. *a*, Haloperidol (10^{-6} M) was administered to a spontaneously firing cell and antagonised the inhibitory effect of DA. *b*, Haloperidol-induced action potentials in a silent but excitable cell when oestradiol-17β (17β-E) was present in the bathing medium. The excitability of the cell was tested by a depolarisation of 0.16 nA.

that DA inhibits both the firing and hormone release supports the hypothesis that CA^{2+}-dependent action potentials are involved in the release of anterior pituitary hormone[7,8]. The mechanism by which DA inhibits the firing of PRL cells is not clearly understood. From our experiments this mechanism appears to be different from that described in central neurones[11], in that no change in the membrane polarisation was observed. Previously, we demonstrated that TRH, which stimulates the secretion of PRL, elicits action potential firing by increasing the membrane resistance without any noticeable depolarisation. These data are in agreement with the finding of Martin *et al.*[12], although Ca^{2+} ions are necessary for the secretion of pituitary hormones, depolarisation is not tightly linked to the release process. Thus, in our experimental conditions, DA appears to act on membrane excitability and resistance of GH3 B6 cells in a way opposite to TRH, although the exact mechanism involved is not known.

The percentage of cells inhibited by DA was dependent on the level of oestrogen in the growth medium. When the cells were grown for at least 24 h in an oestrogen-depleted medium, the percentage of DA-inhibited cells reached 80% (43 out of 54), compared to 40% in normal medium. When oestradiol-17β (10^{-9} M) was added to the recording solution, in all cells tested, DA had no effect on the action potential firing or input resistance ($n = 30$) (Fig. 1*c*). We have previously shown that oestradiol-17β when directly applied to the cell, provokes action potential firing[9]. DA did not inhibit this oestrogen-induced electrical activity. Moreover, haloperidol had a slight effect when added alone, and elicited a short burst of action potentials when oestradiol-17β was present in the recording solution (Fig. 2*b*); haloperidol was ineffective in an oestrogen-free solution. These results are in agreement with the recent observations by Raymond *et al.*[6] that oestrogen has a potent antidopaminergic effect on prolactin release in primary cultures of anterior pituitary cells. Furthermore, it has been recently shown that oestrogen interacts with anterior pituitary DA receptors[14]. The inhibitory effect of DA on action potential firing does not seem to be mediated by a change in membrane polarisation, and so differs from the mechanisms of action of DA so far described[11]. However, at least two classes of DA receptors have been described[15]. The first class of receptors, which increase cyclic AMP synthesis, were found in the caudate nucleus. The second class, which do not increase cyclic AMP synthesis, were described in nigro-neostriatal neurones and mammotroph cells of the pituitary[15]. Recently, Euvrard *et al.*[16] have shown that oestrogen antagonises the inhibitory responses of DA in the striatum. The GH3 B6 cell line may provide a model for the study of some postsynaptic dopaminergic systems, which are of particular interest when considering the role of dopaminergic systems in the modulation of mood and behaviour. Moreover, the model could be useful in the study of the interactions of oestrogen and DA in the control of PRL release at the pituitary level and more so, the action of oestrogen on brain function.

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Benzodiazepine and β-adrenergic receptor ligands independently stimulate phospholipid methylation

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The stepwise methylation of phosphatidylethanolamine by two methyltransferase synthesises phosphatidylcholine in membranes[1]. Although the CDP-choline pathway is the major route for phosphatidylcholine synthesis, recent work in our laboratory has found that the methylation pathway is metabolically highly active. The phospholipid methyltransferases, their substrates and their products are asymmetrically distributed in the cell membrane. Methyltransferase I, located on the cytoplasmic side of the membrane, transfers a methyl group from *S*-adenosylmethionine to phosphatidylethanolamine to form phosphatidyl-*N*-monomethylethanolamine. Methyltransferase II, on the exterior surface of the bilayer, catalyses two additional *N*-methylations to form phosphatidylcholine. The asymmetric distribution of methyltransferases results in the synthesis and translocation of phosphatidylcholine to the exterior bilayer surface[2]. The synthesis and translocation of methylated phospholipid affects many membrane events, such as fluidity[3], coupling of the β-adrenergic receptor with adenylate cyclase[4], the number of β-adrenergic receptors[5], Ca^{2+}-ATPase activity[6], leukocyte chemotaxis[7], mast cell histamine secretion[8] and lymphocyte mitogenesis[9]. We now report that stimulation of a benzodiazepine receptor with anti-anxiety benzodiazepines, and stimulation of the β-adrenergic receptor with β-agonists, increase phospholipid methylation in C_6 astrocytoma cells. Furthermore, simultaneous addition of benzodiazepine and β-adrenergic agonists increases methylation in an additive manner.

Cultured C_6 astrocytoma cells have both β-adrenergic receptors and 'peripheral-type' benzodiazepine receptors[10]. The C_6 astrocytoma avidly binds ^{3}H-diazepam ($K_D \simeq 17$ nM) at a single and saturable class of binding sites. The 'peripheral-type' benzodiazepine receptor of C_6 astrocytoma is differentiated from the 'central-type' benzodiazepine receptor found in the cortex by their differing affinity for two ligands, Ro-5-4864 (a 4-chloro derivative of diazepam) and clonazepam. Ro-5-4864 is potent in displacing ^{3}H-diazepam from the peripheral type site ($K_i = 2.3$ nM), but is ineffective in the 'central-type' site. Clonazepam is ineffective in displacing ^{3}H-diazepam from the 'peripheral-type' site ($K_i = 2{,}000$ nM) whereas it is very potent in the 'central-type' receptor ($K_i = 0.3$ nM). The 'central-type' receptor has been extensively characterised by binding studies[11], biochemical studies[12], electrophysiological studies[13] and interactions with the GABA receptor for γ-aminobutyric acid[14]. The 'peripheral-type' benzodiazepine receptor has been characterised only by ^{3}H-diazepam binding studies.

The effect of benzodiazepines on phospholipid methylation was examined by incubating C_6 astrocytoma cells with [*Me*-^{3}H]methionine. The ^{3}H-methyl-labelled phospholipids were examined by extraction in chloroform/methanol[4]. Separation of ^{3}H-methylated products by TLC demonstrated that 70% of ^{3}H-methyl groups were incorporated into phosphatidylcholine, 5% into phosphatidyl-*N*-dimethylethanolamine and 15% into phosphatidyl-*N*-monemethyl-

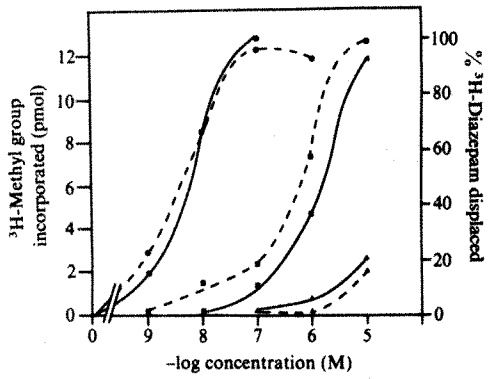


Fig. 1 Benzodiazepine receptor ligands, phospholipid methylation and displacement of ^{3}H-diazepam from the receptor binding site. C_6 astrocytoma cells (41–68 passages) were grown for 4–5 d until confluent in Dulbecco minimal essential media with 4.5 g l^{-1} glucose, 5% fetal calf serum and 4 mM L-glutamine, in FB6-TC multi-dish trays (Linbro) for phospholipid methylation assays (0.5–0.8 mg protein per well), and in Falcon flasks for ^{3}H-diazepam binding assays. The incorporation of ^{3}H-methyl groups into phospholipids was studied by aspirating the media from each well of the multi-dish tray, and adding 1 ml of Media 199 (Microbiological Associates), containing 2×10^{-4} M (±)methionine, and 30 μCi [*Me*-^{3}H]methionine (1 mCi ml^{-1}, NEN) per well. The cells were incubated for 25 min to equilibrate the isotope, and various concentrations of ligand were then added to the wells. After a 30-min incubation, the wells were washed twice with iced 0.9% NaCl. 10% Trichloroacetic acid (TCA), 1 ml, was added, the wells were scraped with a rubber policeman, and the cells transferred by pipette to test tubes. After centrifugation at 27,000*g* for 10 min the supernatant was discarded. Phospholipids were extracted from the TCA precipitate with chloroform/methanol, followed by two washes with 0.1 M KCl in 50% methanol, as previously described[1]. The organic phase, 1 ml, was evaporated in a scintillation vial, resuspended in scintillation fluid, and counted in a spectrometer. The results are expressed as pmol ^{3}H-methyl groups incorporated per 30-min incubation per well, and are the difference in ^{3}H-methyl incorporation between ligand-stimulated and control cells, in at least three separate experiments. Ligands were Ro-5-4864 (●), clonazepam (■) and chlordiazepoxide (▲). After the preincubation, 16.4 ± 4.2 pmol (s.e.m.) of methyl group were found in the phospholipids per mg protein. During the incubation in the absence of ligand for a further 30 min, 24.2 ± 2.1 pmol of methyl group were incorporated per mg protein. Variations occur in different batches of astrocytoma cell cultures, but within the same experiment, were approximately ±5%. ^{3}H-Diazepam binding was measured in membranes prepared from cells. Cells were washed twice with phosphate-buffered saline, *p*H 7.4, and scraped into 10 mM Tris HCl, *p*H 7.0, at 25 °C, homogenised by a Polytron homogeniser, and centrifuged at 40,000*g* for 10 min. The pellet was resuspended in Tris-buffer to a concentration of 2–3 mg protein ml^{-1}. The binding assay was carried out in a final volume of 500 μl, with 100 μl membrane protein, 50 μl of various competing ligands, 4 mM ^{3}H-diazepam (80 Ci mmol^{-1}, NEN) and incubated for 30 min at 4 °C. The reaction was stopped by adding 7 ml iced Tris-buffer and the solution rapidly filtered through a GF/B filter (Whatman). The filter was washed twice with 7 ml iced Tris-buffer, then counted in a liquid scintillation spectrometer. Specific binding of ^{3}H-diazepam is the difference between total binding and nonspecific binding in the presence of 10^{-5} M Ro-5-4864. In a typical experiment, 700 fmol of ^{3}H-diazepam was bound per mg protein and specific binding was 95% of the total binding. Data are expressed as the % displacement of specifically bound ^{3}H-diazepam, and are the mean of duplicate determinations. Ligands tested were Ro-5-4864 (●), clonazepam (■) and chlordiazepoxide (▲).

ethanolamine. Following preincubation of cells with [*Me*-^{3}H]methionine, incubation with benzodiazepine derivatives increased ^{3}H-methyl incorporation into phospholipids (Fig. 1). Methylation was increased by these derivatives in a dose-dependent manner. To determine whether the astrocytoma cells contain the 'central-type' or 'peripheral-type' benzodiazepine receptor, three derivatives of benzodiazepine were used. Their relative potency in increasing phospholipid was characteristic of the 'peripheral-type' receptor (Ro-5-4864>clonazepam> chlordiazepoxide). Furthermore, the potency of several benzodiazepines in stimulating methylation closely matched their potency in displacing ^{3}H-diazepam from the receptor binding site (Fig. 1).

Previous work with rat reticulocytes[4] and HeLa cells[15] demonstrated that stimulation of the β-adrenergic receptor increased the methylation of membrane phospholipids. Similarly, in astrocytoma cells, the addition of the β-adrenergic ligands increased phospholipid methylation. When astrocytoma cells preincubated with [*Me*-^{3}H]methionine were incubated with β-adrenergic agonists, the appearance of methylated phospholipids increased in a dose-dependent fashion. The capacity of these agonists to increase methylation matched for the most part their ability to stimulate adenylate cyclase (Fig. 2). A greater degree of stereospecificity was shown with phospholipid methylation than with adenylate cyclase. Their order of potency in increasing cyclic AMP levels, and in displacing ^{3}H-dihydroalprenolol from the receptor binding site (not shown) was typical for a β_1-adrenergic receptor (isoprenaline > noradrenaline = adrenaline) and exhibited marked stereospecificity ((−)isoprenaline ≫ (+)isoprenaline).

As both the benzodiazepines and the β-adrenergic agonists increase phospholipid methylation, they could be acting on the same methyltransferases or on separate domains associated with the specific receptors. If the β-adrenergic receptor and benzodiazepine receptor increased phospholipid methylation on the same enzyme, stimulating both receptors simultaneously would

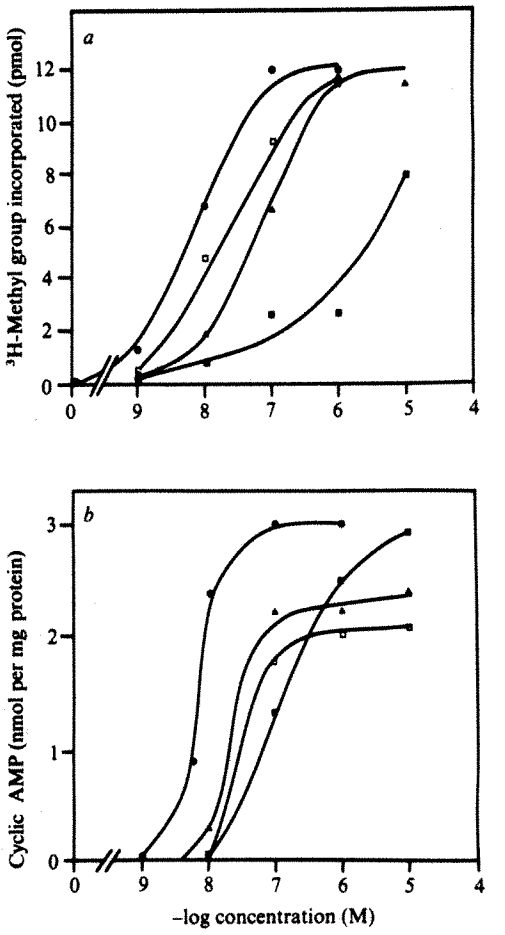


Fig. 2 β-Adrenergic receptor agonists, ^{3}H-methyl groups incorporation into phospholipids, and cyclic AMP levels. *a*, Stimulation of phospholipid by β-adrenergic agonists. C_6 astrocytoma cells were grown in multi-dish trays as previously described. Cells were incubated for 25 min with [*Me*-^{3}H]methionine, and then incubated with various concentrations of agonists for 30 min. The cells were precipitated with TCA and the phospholipids extracted and assayed for ^{3}H-methyl incorporation as described in Fig. 1 legend. The results are expressed as pmol of ^{3}H-methyl groups incorporated per 30-min incubation per well, are the difference in ^{3}H-methyl incorporation between agonist stimulated and control cells in at least three experiments. Agonists used were (−)isoprenaline (●), (−)adrenaline (□), (−)noradrenaline (▲) and (+)isoprenaline (■). *b*, Increased cyclic AMP levels following β-adrenergic receptor stimulation. Intracellular cyclic AMP levels were measured by incubating cells in multi-dish trays for 10 min in various concentrations of agonists. The incubation was stopped by aspirating the media and adding 1 ml of 0.1 M HCl. The HCl aliquot was then lyophilised and redissolved in water. The cyclic AMP content was determined by a protein binding method[17]. The data are expressed as nmol cyclic AMP per mg protein per 10-min incubation, and are the mean of duplicate determinations in two experiments. Ligands tested, and their symbols, are the same as in *a*.

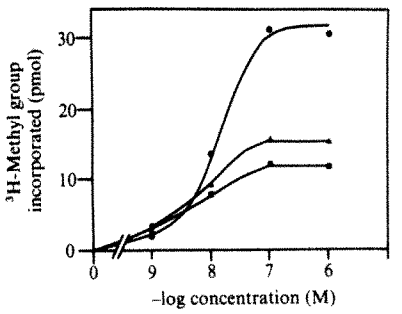


Fig. 3 Effect of simultaneous stimulation of benzodiazepine and β-adrenergic receptors on phospholipid methylation. Following preincubation with [*Me*-^{3}H]methionine, as described in Fig. 1 legend, astrocytoma cells were incubated for 30 min with increasing concentrations of Ro-5-4864 (▲), (−)isoprenaline (■) or both drugs (●). The incorporation of ^{3}H-methyl groups into phospholipids was assayed as described in Fig. 1 legend. The data are the mean of duplicate determinations of three experiments.

increase methylation no more than if either receptor were stimulated maximally. To test this possibility, the cells were treated with increasing concentrations of Ro-5-4864 or isoprenaline, or both drugs together (Fig. 3). Simultaneous stimulation of both benzodiazepine and β-adrenergic receptors increased the appearance of methylated phospholipids in an additive fashion. These results suggest that the methyltransferases are located in separate domains in the vicinity of the respective receptors. Each domain is independently affected by receptor ligands.

Previous work has demonstrated that binding of the β-adrenergic receptor by its agonists increases the methylation of membrane phospholipids[4]. The synthesis of phosphatidyl-*N*-monomethylethanolamine by methyltransferase I increases membrane fluidity[3] and enhances coupling between the β-adrenergic receptor and adenylate cyclase[4], presumably by facilitating lateral mobility of the receptor. The same effects may occur in astrocytoma cells, where β-adrenergic receptor stimulation also increases phospholipid methylation. The activities of both methyltransferases I and II are stimulated by either isoprenaline or benzodiazepines.

Little is known about the physiological or biochemical changes resulting from stimulation of the 'peripheral-type' benzodiazepine receptor, or whether the differences between the 'central' and 'peripheral' receptor reflect an intrinsic difference between these sites. Alternatively, the membrane environment of the receptor may modify its pharmacological properties[16]. The demonstration that benzodiazepine derivatives enhance phospholipid methylation is the first known biochemical event related to this receptor. As phospholipid methylation alters a variety of membrane functions, this observation may provide a rationale for additional studies of whether these 'peripheral-type' receptors have specific functions reported for the 'central-type' receptor.

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How the size of motoneurones determines their susceptibility to discharge

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The susceptibility of motoneurones to discharge by physiological inputs varies inversely with their size[1,2]. This relationship has far-reaching consequences for the control of muscular tension, and also accounts for significant differences in the properties of muscle fibres and motor units[3–5]. The probable basis for the relationship is that the amplitude of excitatory postsynaptic potentials (e.p.s.ps) is also correlated inversely with cell size[6,7], but no satisfactory explanation for this finding is known. We now describe experiments which indicate that afferent impulses invade the relatively simple ramifications of fibres going to small motoneurones more completely than the extensive arborisations going to large cells. This results in activation of a higher percentage of the synaptic endings on small cells, which produces larger e.p.s.ps in them and accounts for their greater susceptibility to discharge.

Experiments were designed to test the following hypothesis. As motoneurones of different sizes reportedly have equal densities of synaptic boutons[8], those with large surface areas must have proportionally more boutons. In particular, if large motoneurones supplying the medial gastrocnemius have more endings from Ia fibres coming from that muscle, this is due, not to the fact that they receive projections from more Ia fibres, but to the more extensive arborisation of individual Ia fibres on these cells, because each motoneurone receives terminals from essentially all Ia fibres coming from its muscle[9]. The more numerous branch points, terminal boutons and *boutons en passant* in extensive arborisations presumably lower the safety factor for propagation[10]. Experimental evidence indicates that

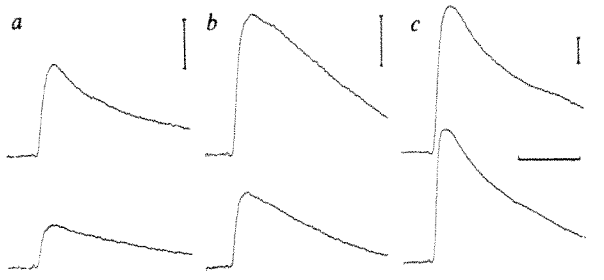


Fig. 1 Examples of aggregate e.p.s.ps recorded from three medial gastrocnemius motoneurones to illustrate the effects of post-tetanic potentiation on cells of different size. Microelectrodes with resistances of 3–10 MΩ were used to record intracellular potentials of motoneurones in cats deeply anaesthetised with sodium pentobarbital. After locating intraspinal regions where antidromic volleys in the axons of MG motoneurones evoked large electrical 'fields', the ventral roots were severed to eliminate motoneurone backfiring which interferes with the recording of their e.p.s.ps. The input resistance of each penetrated cell was obtained by measuring the effects of hyperpolarising and depolarising currents on the amplitudes of antidromic spikes (spike height method)[16]. Eighteen measurements at nine levels of current injection were made. Only cells yielding linear plots of spike height against current were accepted for definitive study. Each of the lower records is the average of 16 monosynaptic, aggregate e.p.s.ps elicited by brief 1 s^{-1} shocks supramaximal for all Ia fibres in the gastrocnemius nerve. After these control responses had been obtained, the muscle nerve was stimulated at 500 s^{-1} for 10 s and e.p.s.ps were again recorded at 1 s^{-1}. The averages of the 3rd to the 18th e.p.s.p. after these tetani are shown in the upper traces. Increases in the size of these e.p.s.ps are of presynaptic origin, being associated with hyperpolarisation of the Ia fibres following a tetanus[15]. The present study suggests that hyperpolarisation enhances local current flow during action potentials, facilitating invasion of terminal arborisations and activating more synaptic knobs. Vertical scale bars, 2.0 mV; horizontal scale bar, 5.0 ms; *IR* values: *a*, 0.3 MΩ; *b*, 1.1 MΩ; *c*, 3.0 MΩ.

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an impulse in a motor axon may fail to invade all its branches[11] and that transmission failures may occur in Ia synapses on motoneurones[12]. It follows that the larger a motoneurone is, the more extensive are the terminal arborisations on it. Therefore, the possibility that impulses in them will die out at certain points will be correspondingly greater and the percentage of Ia endings that are activated by maximal volleys in Ia fibres will be smaller. Anything that facilitates invasion of Ia terminals and activation of synaptic endings should lead to larger aggregate e.p.s.ps and this tendency should be greater in large than small motoneurones.

To test this hypothesis, intracellular recordings were made from motoneurones of anaesthetised cats. First, the input resistance (*IR*) of each motoneurone was measured to provide a reliable estimate of its size[13,14]. Then, the medial gastrocnemius nerve was stimulated at 1-s intervals with brief shocks that set up afferent volleys in all its Ia fibres. The monosynaptic e.p.s.ps evoked by them were recorded and a series of 16 of these responses were averaged electronically and written out on an *x–y* plotter. The muscle nerve was then tetanised at 500 s^{-1} for 10 s and a post-tetanic series of e.p.s.ps was recorded and averaged. Lloyd[15] showed that conditioning tetani of this frequency and duration caused hyperpolarisation of the Ia fibres and a dramatic increase in the amplitude of the monosynaptic reflexes elicited by volleys in them. This 'post-tetanic potentiation' (p.t.p.) fell gradually to control levels within 2–3 min.

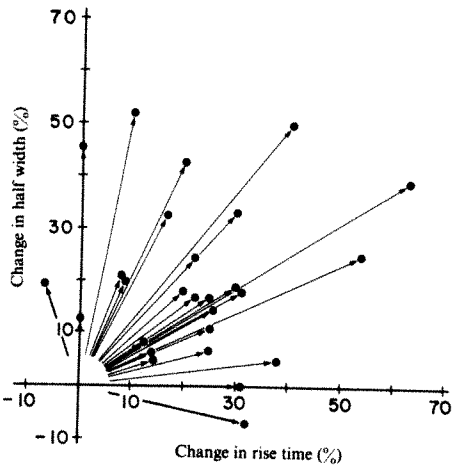


Fig. 3 Plot of changes in rise times of e.p.s.ps against changes in halfwidths following a tetanus to the muscle nerve at 500 s^{-1} for 10 s. Arrows indicate directions of change. The time courses of e.p.s.ps in these experiments were sometimes altered by afferent impulses in group II fibres, which also project monosynaptically to motoneurones[17]. The data plotted here were obtained from records where there was no evidence of group II inputs, but the possibility of a contribution from them could not be eliminated entirely.

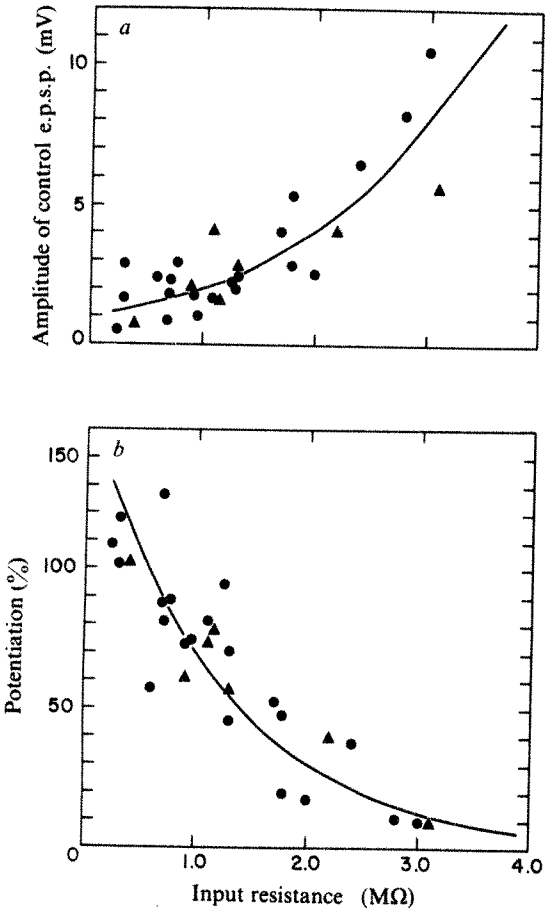


Fig. 2 Relationship between input resistances of motoneurones (in MΩ) and amplitudes of their aggregate e.p.s.ps (in mV) before (*a*) and during (*b*) post-tetanic potentiation. Data were pooled from nine experiments. Measurements from a few semitendinosus motoneurones (▲) are plotted with those from medial gastrocnemius (●) to illustrate the essential similarity of the relationship for both species of motoneurone. Comparison of *a* and *b* indicates that motoneurones with the largest control e.p.s.ps exhibited the least potentiation and those with the smallest control e.p.s.ps were potentiated the most, as our hypothesis predicts. The exponential curves through the two sets of data points were plotted by the method of least squares.

Figure 1 shows typical effects of p.t.p. on the e.p.s.ps of three motoneurones of different sizes. The lower traces in the three pairs of records illustrate that the average size of the aggregate e.p.s.ps before potentiation varied directly with the *IR* of the motoneurone (see values in legend to Fig. 1). This confirmed previous observations[7]. The upper traces are the averages of 16 e.p.s.ps following the tetanus. The per cent potentiations in *a*, *b* and *c* were 123, 92 and 11, respectively.

Figure 2*a* plots the relationship between input resistance and amplitude of control e.p.s.ps in data pooled from nine experiments. Figure 2*b* illustrates the way in which per cent potentiation was related to input resistance in the same experiments. As our hypothesis predicts, the greatest potentiation occurred in motoneurones with the lowest *IR*, that is, in the largest cells. The least potentiation and the largest control e.p.s.ps were found in motoneurones with the highest *IR* values. Less branching would be expected in Ia fibres approaching these small cells and few inactive endings should be available to increase synaptic input during p.t.p. It has generally been assumed that p.t.p. is due to release of more transmitter from a fixed number of active endings. This assumption, however, would predict that the effect of p.t.p. is independent of cell size and is no longer tenable.

If our hypothesis is correct, p.t.p. might be expected to cause small increases in the conduction time required for Ia volleys to invade additional terminals and to activate more synaptic knobs. The flow of synaptic currents at the various endings might be less synchronous, that is, more prolonged due to these delays[10], but it is difficult to predict the effects of p.t.p. on the time course of e.p.s.ps without precise information about the geometry of the terminal arborisation of Ia fibres. Figure 3 plots the changes in rise time in e.p.s.ps against the changes in their halfwidths during p.t.p. In general, rise times and halfwidths increased during p.t.p. as predicted, but the magnitudes of these changes do not seem to be related (see Fig. 3 legend).

These experimental findings are consistent in all respects with our proposed hypothesis. They suggest that invasion of Ia terminals is a graded process that is generally more complete in the terminal arborisations on small cells because they have fewer branch points. The percentage of Ia boutons that are activated by afferent impulses depends on this process. The surface area that a motoneurone presents to approaching Ia fibres may influence their tendency to branch and, thus, indirectly determine its own susceptibility to discharge.

Activation of pre-existing but inactive synaptic endings, which our findings imply, provides the functional equivalent of

new connections. Experiments to determine whether a similar mechanism, with a more prolonged time course, is used in the circuits involved in memory and learning might offer promising leads.

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Single ionic channels observed in tissue-cultured muscle

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The extracellular patch clamp technique developed by Neher *et al.*[1] to record the responses of single channels in skeletal muscle has provided firm evidence for the two-state nature of the conductance event in nicotinic endplate channels. We report here the use of the extracellular patch technique to record single-channel responses from tissue-cultured chick skeletal muscle cells. The temperature dependence of channel conductance and gating kinetics shows no evidence of discontinuous behaviour between 17 and 37 °C.

The ability to record single-channel responses makes it possible to investigate properties of excitable cells which are otherwise inaccessible. Fischbach and Lass[2], have reported that in a tissue-cultured preparation of chick 'myoballs', the single-channel conductance (as estimated by fluctuation methods) increased with temperature to about 20 °C, and then remained constant at about 30 pS. The channel open time, as estimated by the half-power frequency of the fluctuation power spectrum, did not show any abrupt change with temperature. Their data present two simple alternative interpretations: (1) that there are two or more populations of channels, high conductance and low conductance, which change proportions as a function of temperature; or (2) that the whole population of channels shows identical, discontinuous, responses to temperature. Using kinetic techniques, the first alternative cannot be distinguished from the second as the populations are apparently kinetically indistinguishable. However, the problem could be solved if the response of the individual events could be resolved.

Using 100 nM suberyldicholine as an agonist, we measured single-channel responses in cells at rest and in cells in microelectrode voltage clamp conditions. Suberyldicholine was chosen as the agonist as its channel open time is longer than that for other agonists. A typical record of channels observed in an unclamped cell is shown in Fig. 1. The salient features of the record are similar to the results reported for frog muscle[1,3]. Two-state current pulses of inward current, shown as downward deflections, are the most striking features. We calculated the single-channel conductance assuming a reversal potential of

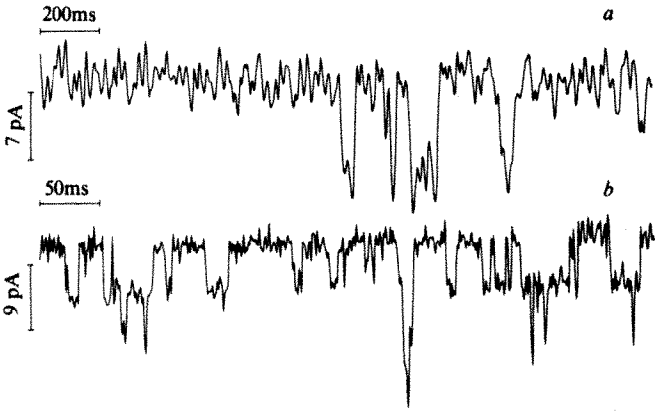


Fig. 1 Digitised membrane current traces showing pulse-like agonist-activated current flow through single channels. Downward deflections indicate inward current. Both records were filtered at a bandwidth of 280 Hz. *a*, 23.5 °C, membrane potential −49 mV, at rest. *b*, Different cell, 38 °C, membrane potential −102 mV, under voltage clamp. Cultures were prepared from 10–12-day-old Leghorn embryos using mechanical disaggregation in divalent ion-free phosphate-buffered saline[4]. The cells were plated on gelatin coated plastic tissue culture dishes in Dulbecco's modified Eagle's medium containing 10% heat inactivated horse serum and 2% embryo extract. The cells were treated with 10 μM cytocine arabinoside to prevent overgrowth of fibroblasts[14]. For experiments, the dishes were filled with HEPES-buffered Hank's saline containing 10% heat inactivated horse serum covered with a layer of mineral oil, and placed on the temperature-controlled stage of an inverted, phase contrast microscope. Single-channel records were made either on cells at rest with an intracellular microelectrode to record potential or in voltage clamp conditions using a standard two microelectrode voltage clamp. Extracellular patch recording pipettes were made according to techniques described by Neher *et al.*[1]. The pipettes were filled with Hanks' saline containing 50–100 nM suberyldicholine. Pipette resistances varied from 1–5 MΩ in solution, increasing to 3–20 MΩ when placed against the cell surface. The patch recording system was essentially the same as described previously[1] with the addition of an automatic drift compensation circuit (E. Neher, unpublished) and an internal calibration circuit. Data were recorded on analogue magnetic tape for later analysis.

0 mV and a linear current voltage relationship[4,5]. At 22 °C we calculated a mean channel conductance of 60 pS (compared with 48 pS observed by Jackson and Lecar[5]). Working with frog extrajunctional receptors, Neher and Sakmann[6] estimated the single-channel conductance to be about 15 pS at 8 °C. Even if a Q_{10} of 1.5 is assumed, the conductance is significantly lower than the values reported here for homeotherms. In adult rat, Sakmann[7] has reported a channel conductance at 22 °C of 34 pS at the endplate, and 25 pS extrajunctionally. The tissue-cultured preparations seem to have a larger channel conductance than the adult tissues.

The open channel duration is an exponentially distributed random variable (Fig. 2). The mean channel open time was estimated as the time constant of an exponential least squares fit to the duration histogram. Using suberyldicholine, the mean time constant of channel closing was 13 ms at 24 °C. This should be compared to 3 ms observed in tissue-cultured rat muscle with carbachol as an agonist[5]. Using fluctuation and voltage jump relaxation methods, Neher and Sakmann[8] have estimated the mean channel open time at 18 °C in the frog to be 0.5 ms with carbachol and ~4 ms with suberyldicholine. Thus, it is clear that carbachol as an agonist is less efficient than suberyldicholine in keeping channels open. At the same temperature, both carbachol and suberyldicholine appear to keep channels open for relatively longer periods in homeothermic preparations than in poikilothermic preparations. When examined over a range of temperatures, the single-channel conductance and open time behave simply (Fig. 3). The variation of channel conductance with temperature is shown in Fig. 3*a*. Channel conductance

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increases with increasing temperature with a Q_{10} of 1.3. The channel conductance variation with temperature is similar to the Q_{10} of 1.7 reported earlier from fluctuation analysis using acetylcholine[4]. Contrary to the results reported by Fischbach and Lass[2], we see no levelling off of the conductance at high temperatures, or any evidence of discontinuous change in conductance with temperature. The single-channel results are unambiguous in showing a high conductance at body temperature. The temperature dependence of the mean channel open time is shown in Fig. 3*b*. The enthalpy of activation, as calculated from the slope of the regression line, was 4.4 kcal mol^{-1}. If we combine data from fluctuation measurements[4] and the present work, a curious pattern emerges whereby the activation enthalpy of the closing rate is inversely correlated with the open time. This may reflect entropic contributions to the activation energy for closing or perhaps a more complicated interaction of the rates for agonist binding and channel open-closed isomerisation.

Some records showed what appeared to be multiple gating of a single channel (Fig. 4). In these records the mean opening rate was low (duty cycle = 0.03) and hence there was a low probability of finding more than one channel open at a time. Nonetheless, we observed many events similar to those shown in Fig. 4 in which a channel apparently turns on and off several times before closing 'for good'. This burst-like behaviour is similar to that reported by Patlak *et al.*[9] for desensitised channels and by Neher and Steinbach[10] for endplate channels treated with local anaesthetics. As the opening rate and the agonist concentration were low, high probability agonist binding does not seem to be the cause of the multiple gating events. Perhaps suberyldicholine as a bivalent agonist, is able to block the same channels it activates[11], or the agonist dissociation rate is comparable to the channel opening rate so that several open–closed cycles may take place before agonist dissociation[12]. Further work needs to be carried out to elucidate the mechanism of this repetitive behaviour.

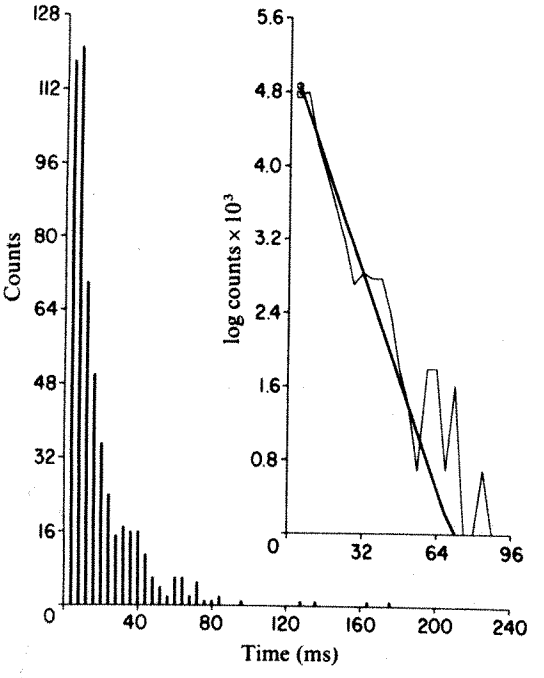


Fig. 2 Histogram of single-channel durations. Figure 1*a* is a typical segment of the data used to construct the histogram. Inset shows the same data plotted semilogarithmically and fitted with a single exponential (heavy line) by a least squares fit routine. Both amplitude and duration histograms were calculated from the raw data by an automatic pattern recognition program (F.S. and N. Barkakati, in preparation).

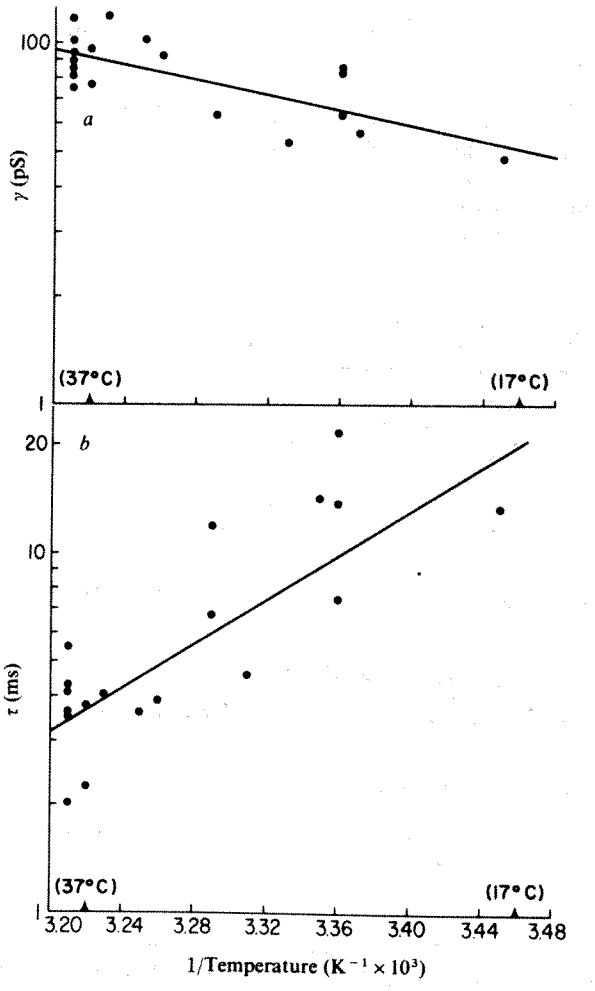


Fig. 3 Arrhenius plot of the single-channel conductance (*a*) and mean channel open time (*b*). Each point represents the results of measurements on an individual cell. *a*, Single-channel currents used to calculate conductance were obtained from amplitude histograms in accordance with a model which predicts the shape of the histogram and takes into account the fact that the wall thickness of the patch pipette is not negligible (channels which lie under the rim of the pipette do not contribute a full share of current to the observed record (F.S. and N. Barkakati, in preparation). The solid line is a least squares regression with a slope equivalent to an enthalpy of activation of 2 kcal mol^{-1}. *b*, Mean open times from individual cells were obtained from duration histograms. The line, which represents a least squares regression fit to the data, corresponds to an enthalpy of activation of 4.4 kcal mol^{-1}.

This work demonstrates that single-channel recording techniques can be easily extended to a wider variety of preparations than previously reported. More specifically, we find no anomalous temperature dependence of channel conductance. Results presented here were obtained on myotubes not treated with vinblastine, so that vinblastine treatment cannot account for the discrepancy between the results of Fischbach and Lass[2] and the results presented here. The origin of the discrepancy remains obscure, but we are reticent to ascribe the differences to

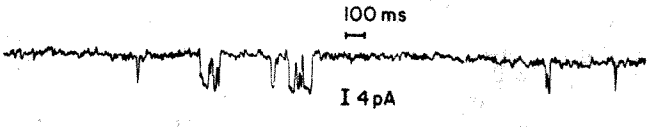


Fig. 4 Chart recording of membrane current illustrating the occurrence of apparent multiple gating of a single channel (see text for details). Temperature, 27 °C; bandwidth, 100 Hz.

the slight variations between the preparations, as these results agree reasonably well with the earlier results obtained with fluctuation analysis on vinblastine-treated cells, as well as with earlier data on myotubes using acetylcholine as an agonist[13].

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Single postsynaptic channel currents in tissue cultured muscle

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Some of the most compelling evidence for the existence of ionic channels in cell membranes comes from direct recording of quantised current jumps generated by the opening and closing of individual channels. Single-channel jumps have been extensively studied for lipid bilayer membranes doped with various channel-forming additives[1]. Recently agonist-induced single-channel currents were detected in denervated frog muscle by use of extracellular electrodes, which can isolate the current from a small area of membrane[2]. The current jumps provide a means for the direct test of many of the inferences about ionic channels which have come from electrical noise analysis[3,4]. In this report we present measurements of single-channel currents induced by the agonist carbamylcholine in tissue-cultured mammalian muscle. These measurements confirm the earlier noise studies on tissue culture preparations[5,6]. Recordings of single-channel currents induced by the agonist, suberyldicholine, in avian muscle are presented by Nelson and Sachs[7].

The methods used for single-channel recording have been described by Neher *et al.*[8]. A small ($\sim 1\ \mu m^2$) patch of cell surface is isolated with a specially fabricated extracellular glass microelectrode. A low-noise circuit amplifies the current through this patch of membrane. The signal is then recorded on a digital oscilloscope and can be stored on tape for later analysis. Dissociated embryonic rat muscle was grown according to the procedure of Nelson *et al.*[9].

Figure 1 shows channels recorded from a cell held at a membrane potential of -90 mV. From such recordings, the single-channel conductance and the open-channel durations are measured directly. Figure 2*a* shows a histogram of the distribution of open-channel lifetimes. The number of channels remaining open for a time greater than T is plotted against T. The semilogarithmic plot of open-channel durations fits well to a single-exponential distribution, consistent with the notion that the closing of the channels is a process having a Poisson distribution. The mean channel open time at a temperature of 22 °C is 3.2 ms.

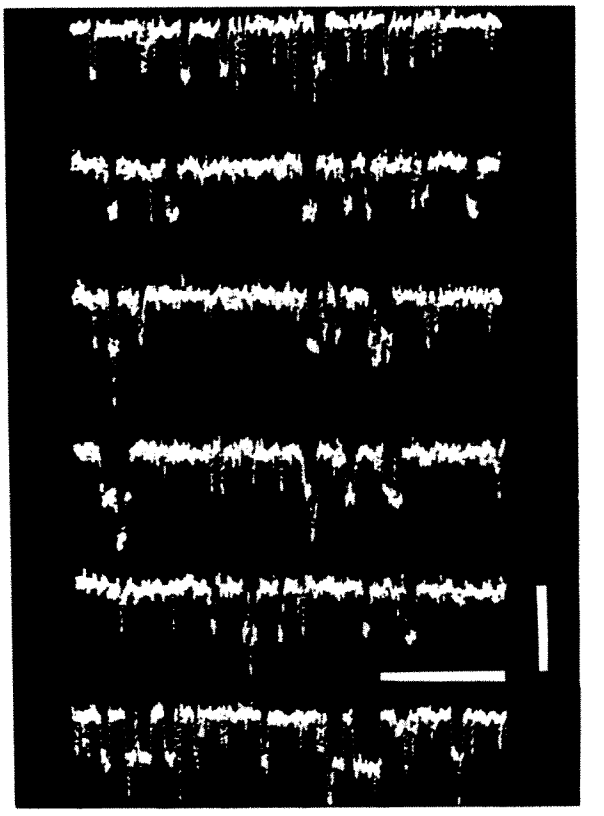


Fig. 1 Channel records made from cultured rat muscle at 22 °C. The muscle was bathed in Dulbecco's phosphate-buffered saline, with tetrodotoxin added to prevent spontaneous contraction. The currents were recorded from a thick-walled glass micropipette fabricated with a deFonbrune microforge. This micropipette was filled with saline and 1.0 μM carbamylcholine. Its resistance was 3 MΩ and increased to 40 MΩ when pushed against the cell surface. The cell was hyperpolarised to -90 mV using two intracellular electrodes, one to pass current and the other to record the cell membrane potential. Scales: horizontal, 100 ms; vertical, 10 pA.

The mean single-channel current at -90 mV is 4.4 pA, indicating that the unit channel conductance is 49 pS (assuming a reversal potential of zero for the postsynaptic channel). The standard deviation obtained from the channel current amplitude distribution is 0.8 pA. The two main reasons for this spread are the intrinsic current noise of the wide bandwidth amplifier needed to record the short-lived events and the recording of undersized current jumps from channels situated beneath the annulus of the pipette.

As a check on the straightforward but tedious determination of individual channel amplitudes and durations, we also used simple automated procedures to obtain these parameters. Amplitude histograms were made directly from the data using a computer program. Figure 2*b* inset shows a current amplitude histogram using 4,096 points spaced 200 μs apart. The direct histogram shows three peaks, a large gaussian distribution of baseline noise centred at zero, a second peak about the single channel amplitude, and a third smaller peak at twice the single-jump amplitude. The channel current can be estimated from the displacement of the secondary peaks without the necessity of analysing individual events. The weighting of events is different here than for the direct measurement, as a long-lived event will contribute more points to the histogram than a shorter event. Using the amplitude histograms, we obtained a single-channel current–voltage curve (Fig. 2*b*). The plot of current peaks versus applied membrane potential is linear over the voltage range measured. From the slope of the line, we obtain a unit conductance of 48 ± 0.4 pS.

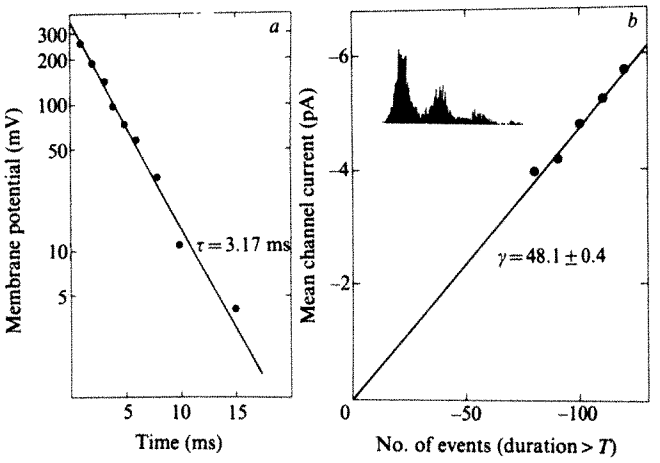


Fig. 2 *a*, A semilogarithmic plot of the number of events with duration greater than time T plotted against T shows the exponential probability of channel open time. The line is a least squares fit to the data. *b*, Single-channel current-voltage curve. Abscissa gives holding potential with increasing hyperpolarisation to the right. Ordinate shows inward current as determined from the second peak of current histogram. Inset, a typical current histogram, taken at a holding potential of −100 mV. The histogram is determined from a record of 4,096 points taken at 200-μs intervals; the horizontal distance between the first two peaks is 4.8 pA and the vertical displacement is proportional to the number of points with a given amplitude.

To analyse channel lifetimes automatically, a Fast Fourier Transform program was used to determine the power spectra of digitised records similar to those shown in Fig. 1, but usually with a higher density of channel events. The power spectra are lorentzian, as expected from the underlying Poisson random process. The mean channel durations determined from the cutoff frequencies of the noise spectra were in agreement with the values determined from the pulse-duration histograms. Thus, spectral analysis methods can be used as a convenient method to analyse stationary records of single-channel jumps.

Single-channel measurements seem to be feasible for a variety of tissue-cultured excitable cells, and the patch-electrode method allows one to observe the ion channel activity in an area of a heterogeneous membrane of less than 5 μm^2. Preliminary measurements carried out in collaboration with C. N. Christian indicate that the distribution of channel density determined with an extracellular patch electrode coincides with that obtained by observing the same cells in a fluorescence microscope using rhodamine labelled α-bungarotoxin[10].

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Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn*9*

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The transposable genetic element Tn*9* consists of two direct repeats of the insertion sequence IS*1* flanking a region of 1,102 base pairs which determines chloramphenicol resistance[1,2]. Transposition of Tn*9* leads to the duplication of a 9-base pair sequence which preexists at the site of insertion. One copy of this sequence is found at each end of the inserted element[3]. The chloramphenicol resistance determined by Tn*9*, and by various other R plasmids, is due to the synthesis of the enzyme chloramphenicol acetyl transferase (CAT)[4,5]. This enzyme catalyses the formation of acetylated derivatives of chloramphenicol which are inactive as inhibitors of protein synthesis[5]. By using the chain termination technique of DNA sequencing, we have now determined the nucleotide sequence of the 1,102 base pair region between the directly repeated IS*1* sequences in the bacterial transposon Tn*9* (encoding chloramphenicol resistance). The amino acid sequence of CAT predicted from the nucleotide sequence is identical to that determined by Shaw and coworkers[6]. An analysis of the sequence suggests that the internal 1,102 base pair region is not directly involved in transposition.

Restriction endonuclease analysis of bacteriophage P1Cm DNA demonstrated that Tn*9* contained unique *PstI* cleavage sites within the directly repeated IS*1* sequences but no *PstI* sites within the chloramphenicol resistance determinant[7]. Cleavage of bacteriophage P1Cm with *PstI* generated a fragment of 1,870 base pairs. This fragment contained the 1,102-base pair region encoding the structural gene for CAT flanked by 588 base pairs from the right end of IS*1* and 180 base pairs from the left end of IS*1*. The 1,870-base pair fragment was cloned in the *PstI* site of plasmid pBR322 generating pDV801 which served as the source of DNA in these studies (Fig. 1). A restriction map of the 1,102-base pair region is presented in Fig. 2.

To determine the nucleotide sequence of the 1,102-base pair region of Tn*9* bounded by the IS*1* sequences we used the rapid dideoxy chain termination technique developed by Sanger *et al.*[8]. Two methods were used to prepare the required single-stranded templates. One of these is exonuclease III digestion of double-stranded DNA[9]. This enzyme removes nucleotides from the 3′ ends of double-stranded DNA. Therefore, exonuclease III digestion of a linear double-stranded molecule will generate a single-stranded region which may be used as a template in the primed synthesis reaction (Fig. 3). A typical gel using templates prepared in this manner is shown in Fig. 4. The other method of obtaining single-stranded templates involves molecular cloning in the single-stranded filamentous bacteriophage M13 (ref. 10). Infection of susceptible strains (sex-factor carrying strains of *Escherichia coli* K12) with M13 results in the establishment of a carrier state in which the double-stranded replicative form (RF) of M13 is maintained at about 300 copies per cell, while mature phage, containing one of the complementary strands ((+) strand), is extruded into the medium[11,12]. It has recently been demonstrated that foreign DNA can be inserted into and stably maintained in the M13 RF (refs 10, 12–14). Since the limit to the size of the DNA packaged by filamentous phage appears to be very large, the inserted DNA can be packaged as single-stranded DNA covalently linked to the phage DNA. This single-stranded DNA can then be isolated and used as a template for the dideoxy sequencing method. By cloning the DNA in both

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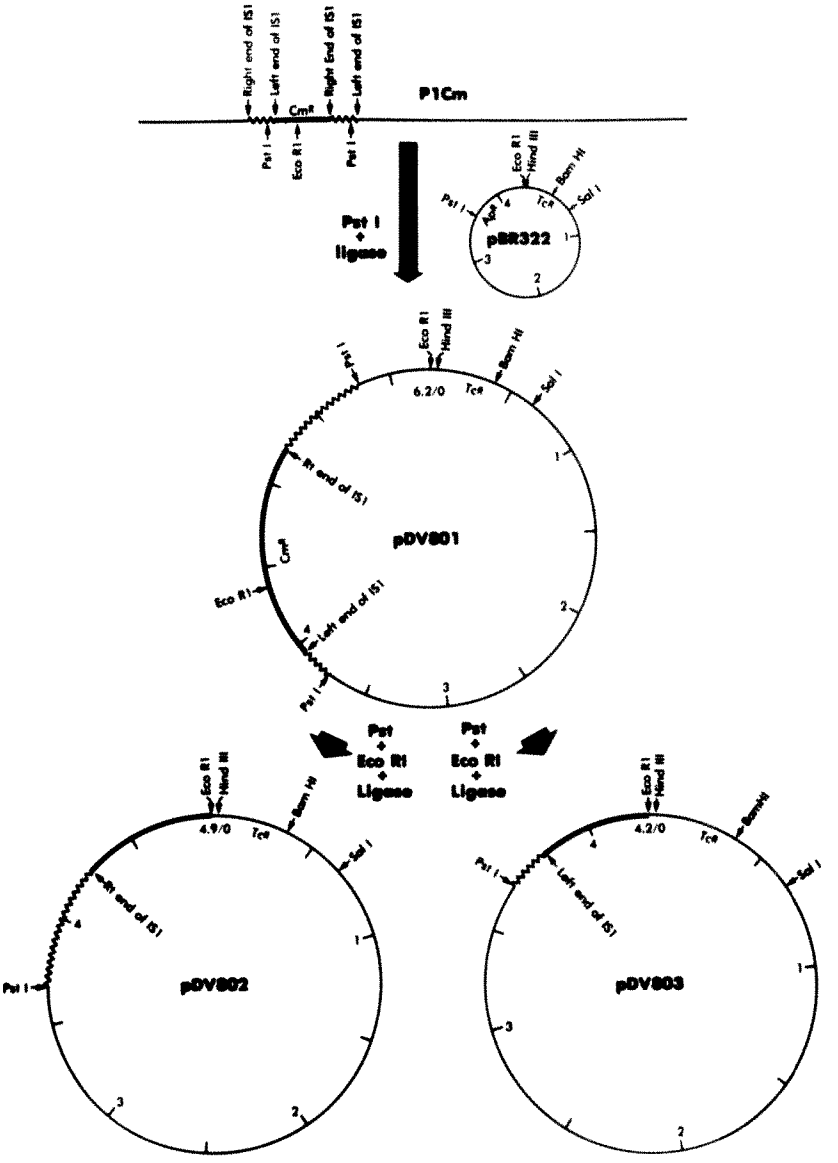


Fig. 1 Schematic representation of plasmid constructions. The derivation of pDV801 is shown and was previously described[36]. pDV802 and pDV803 were constructed by digestion of pDV801 with *Pst*I and *Eco*RI, treatment with T4 polynucleotide ligase, and transformation of *E. coli* C600 using published procedures[37]. The plasmids carried by several tetracycline-resistant, chloramphenicol sensitive strains were isolated and the fragments of Tn*9* they contained determined by digestion with *Eco*RI plus *Pst*I. pDV802 carries a 1,254 base-pair fragment containing the right end of IS*1* while pDV803 carries a 616 base-pair fragment containing the left end of IS*1*. Wavy lines indicate IS*1* sequences. The numbers within the circles refer to distances in kilobases.

orientations, either of the complementary strands can be isolated (Fig. 5*a*). An example of a sequencing gel using M13 templates is shown in Fig. 5*b*.

In initial sequencing experiments, the exonuclease III method was used to prepare templates. During this work the M13 method became available and was used in completing the sequence. For both methods, DNA fragments generated by restriction endonuclease digestion of the 1,870-base pair fragment of Tn*9* were used as primers (Fig. 2).

Using these techniques we have determined the nucleotide sequence of the 1,102-base pair region of Tn*9* bounded by the directly repeated IS*1* sequences (Fig. 6). In most regions both of the complementary strands were sequenced several times (Fig. 2). Amino acid sequence data of the CAT gene allowed further confirmation of the sequence as shown in Fig. 6 (ref. 6).

We have also sequenced approximately 200 base pairs within IS*1* including 40 base pairs at the right end and 70 at the left end. The sequence of these regions is identical to that determined by Ohtsubo and Ohtsubo[15] and Johnsrud[16].

Three unique restriction endonuclease cleavage sites are located within the CAT coding sequence. These are an *Eco*RI site (nucleotide 437), a *Bal*I site (nucleotide 704), and a *Pvu*II site (nucleotide 337) (Fig. 6). These sites are potentially useful for DNA cloning since insertion of DNA into these sites should lead to the loss of chloramphenicol resistance, thereby simplifying the screening of recombinants. Two cloning vehicles which carry the CAT gene, pBR325 and pACYC184, have been constructed and used as *Eco*RI vectors[17,18]. pBR325, however,

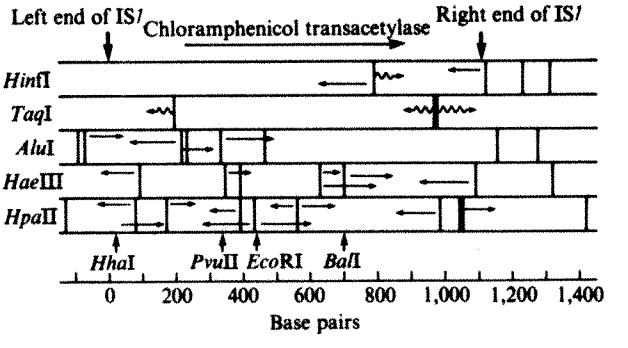


Fig. 2 Map of restriction endonuclease sites and sequencing strategy of the 1,102 base-pair region of Tn*9* contained between the IS*1* sequences. Positions of the endonuclease cleavage sites were determined using the technique described by Smith and Birnsteil[38] and by analysis of multiple digestion products as previously described[37]. Restriction endonucleases were obtained from New England Biolabs. Cleavage with restriction endonucleases *Hae*III, *Hpa*II, *Alu*I, *Taq*I and *Hha*I was carried out in H buffer which contains 6.6 mM Tris-HCl, *p*H 7.5, 6.6 mM $MgCl_2$, 6.6 mM NaCl, 5 mM dithiothreitol. Cleavage with restriction endonucleases *Pst*I, *Eco*RI and *Hin*fI was in H buffer plus 50 mM NaCl. All reactions were at 37 °C with the exception of *Taq*I which was at 60 °C. The position of the *Bal*I site is inferred from the nucleotide sequence. Vertical lines indicate cleavage sites for the endonucleases shown at the left. Arrows show the direction and extent of sequencing runs. Wavy arrows indicate sequencing runs performed using the Maxam and Gilbert technique[40]. The left and right ends of IS*1* are indicated.

has an additional *Pvu*II site as well as an additional *Bal*I site. These sites would have to be removed to make appropriate cloning vehicles for use with these restriction endonucleases.

Since the nucleotide sequences surrounding these sites are now known, they are also potentially useful for the formation of fused proteins. These fused proteins would then be under the control of the CAT promoter. It has recently been demonstrated that the CAT gene carried by pBR325 can be expressed in *Saccharomyces cerevisiae* (J. Cohen and R. Needleman, personal communication). Therefore, it would be feasible to test the expression of these fused proteins in yeast as well as in *E. coli*.

Analysis of the nucleotide sequence reveals an uninterrupted coding sequence for 219 amino acids beginning with the AUG initiator codon at nucleotide 224 and terminating with the nonsense codon UAA at nucleotide 881. This amino acid sequence is identical to that determined by Shaw *et al.* for the Type I CAT isolated from strains carrying plasmid JF66b (see the accompanying paper)[6].

Shine and Dalgarno[19] suggested that ribosome recognition of mRNA involves complementary base-pairing between the 3′ end of the 16S rRNA and the 5′ end of mRNA. Such complementarity has been confirmed by sequence analysis of a number of different genes[20–22]. Two such sequences are found preceding the initiation codon for the CAT gene. These are AGGAG (nucleotides 205–209) and TAAGGA (nucleotides 211–216). Based on its spacing from the AUG initiator codon, the sequence TAAGGA is most likely involved in ribosome recognition. However, it is possible that the AGGAG sequence might also have some role.

The sequence data in Fig. 6 show that the amino terminal codon of the CAT gene is located 224 nucleotides from the left end of IS*1*. Assuming that the CAT promoter is not in IS*1*, these 224 nucleotides encode the regulatory functions of the CAT gene. The level of expression of the CAT gene is regulated by the cyclic AMP receptor protein (CAP) and cyclic AMP[23,24]. The promoter regions of the lactose and galactose operons which are also regulated by CAP plus cyclic AMP contain a sequence, referred to as the CAP site, which precedes the start point of transcription (for reviews see refs 25, 26). This region presumably interacts with the CAP–cyclic AMP complex stimulating the initiation of transcription. Biochemical and genetic data have demonstrated that the CAP site in the *lac* promoter contains the sequence GTGAG----CACTC which is involved in binding the CAP–cyclic AMP complex[25]. Similar sequences are found in the promoter regions of the galactose and *araBAD* operons[27,28]. Inspection of the DNA sequence between the left end of IS*1* and the start of the CAT coding sequence reveals a similar sequence GTGA----TCAC (Fig. 6, nucleotides 29–43). By analogy with *lac*, *gal* and *araBAD* this region may be

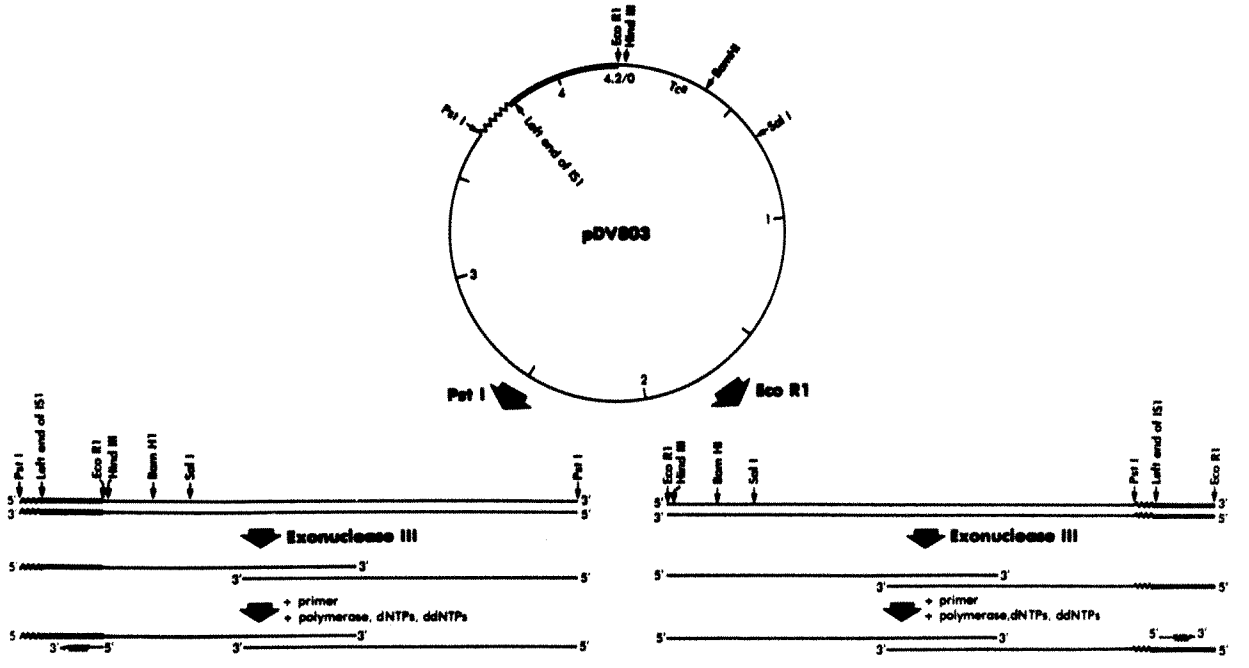


Fig. 3 Exonuclease III preparation of templates for the primed synthesis reactions. The most useful templates prepared by exonuclease III treatment were those containing the DNA to be sequenced at the end of the molecule. Therefore, plasmids to be used for preparing exonuclease III templates should contain unique restriction cleavage sites adjacent to the region to be sequenced. To prepare such plasmids we subcloned the fragments of Tn*9* generated by digestion of pDV801 with *Eco*RI plus *Pst*I. The two plasmids derived from this cloning, pDV802 and pDV803, have unique *Pst*I and *Eco*RI sites (Fig. 1). The use of one of these plasmids, pDV803, to prepare single-stranded templates is outlined above. Cleavage with *Pst*I followed by exonuclease III digestion exposes one strand of Tn*9* while cleavage with *Eco*RI followed by exonuclease III treatment exposes the opposite strand. By utilising both of these templates the sequence of the complementary strands could be determined. DNA prepared in this manner was suitable as a template using restriction endonuclease generated fragments less than 100 base pairs as primers. Larger fragments were not as useful since they must be cleaved with restriction endonucleases from the extended chains before gel electrophoresis. This cleavage step was necessary to obtain chains small enough (less than about 200 base pairs) to be resolved on the sequencing gel. However, cleavage of primers when using exonuclease III prepared templates led to artefactual bands in all lanes of the gel, probably due to residual double-stranded regions in the DNA template which could either be nick-translated or whose 3′OH ends could act as self-primers. In either case, restriction endonuclease digestion would release radioactively labelled chains. If these chains were of a size which would migrate within the resolving limits of the sequencing gel, they would appear as bands in all channels. Smith[9] found that this problem could be eliminated by more extensive treatment with exonuclease III. However, in our hands, longer exonuclease digestion led to degradation of the DNA and loss of template activity. Although such degradation of the single-stranded DNA might be due to the endonuclease III activity known to be associated with exonuclease III, this is unlikely since the endonuclease II activity has been shown to be specific for damaged DNA[39]. Another possibility is that some other nuclease was present in the preparation of exonuclease III used in these experiments. Exonuclease III can also be used for the preparation of primers (R. J. Roberts, personal communication). Double-stranded restriction endonuclease generated fragments were exhaustively digested with exonuclease III, and the products of this reaction used as primers with the M13 templates described in Fig. 5. Analysis of sequences determined by this technique demonstrated that the double-stranded fragment had been reduced to single-stranded primer of approximately 15 bases. If the double-stranded fragments treated with exonuclease III for use as primers are small (less than the resolving limits of the sequencing gel), sequences adjacent to the fragment can also be determined.

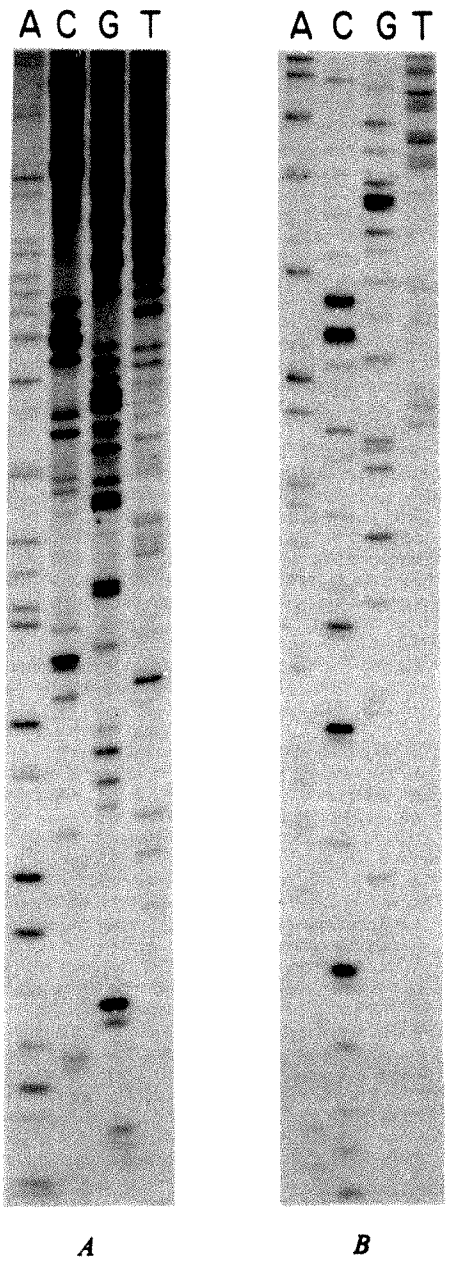


Fig. 4 Autoradiogram of a sequencing gel using exonuclease III treated templates and primers. Plasmid DNA was isolated as previously described and purified by isopycnic centrifugation in CsCl-ethidium bromide gradients[37]. The DNA was further purified by velocity sedimentation in a 5–20% neutral sucrose gradient containing 1 M NaCl, 0.1 M Tris-HCl, *p*H 7.0. 100–200 μg of DNA was layerd on a 5 ml gradient and centrifuged for 3.5 h at 40,000 r.p.m. in a SW50.1 rotor. Peak fractions containing the DNA were pooled and the DNA precipitated by the addition of 2.5 volumes of 95% ethanol, pelleted by centrifugation (40,000 r.p.m., 20 min), resuspended in distilled water and stored frozen at −20 °C. For exonuclease III treatment, 10 μg of DNA was digested with either *Pst*I or *Eco*RI (H buffer + 50 mM NaCl) in 25 μl followed by incubation for 10 min at 65 °C to inactivate the restriction endonucleases. The DNA was then dialysed for 2 h against 200 ml of exonuclease III buffer (50 mM Tris-HCl, *p*H 8.0, 1 mM $MgCl_2$, 1 mM dithiothreitol (DTT)). The volume was adjusted to 50 μl and 10 units of exonuclease III (New England Biolabs, 1 unit per μg DNA) was added. After incubation for 45 min at 37 °C, an additional 10 units of exonuclease III was added and incubation was continued for 30 min at 37 °C. Exonuclease III was inactivated by a 15 min incubation at 65 °C and the DNA was stored at −20 °C. For use as a template, 0.1 pmol of exonuclease III-treated DNA was mixed with 0.5 pmol of restriction fragment primer prepared as described below in a final volume of 10 μl containing H buffer. This mixture was then sealed in a glass capillary, boiled for 3 min and hybridised at 68 °C for 30 min. 2 μl of this mixture was used in each reaction as described below. Extension with *Pol*I (Klenow fragment, New England Biolabs) in the presence of deoxynucleotide triphosphates and dideoxynucleotide triphosphates was performed essentially as described by Sanger *et al.*[8]. Reactions were carried out in 1.5 × H buffer containing 0.5 μM [α-^{32}P]dATP (400 Ci mmol^{-1}, Amersham Radiochemicals) and nucleotides at the following concentrations:

ddA: 35 μM dCTP; 35 μM dGTP; 35 μM dTTP; 50 μM ddATP
araC: 3.5 μM dCTP; 35 μM dGTP; 35 μM dTTP; 5000 μM araCTP
ddC: 3.5 μM dCTP; 35 μM dGTP; 35 μM dTTP; 200 μM ddCTP
ddG: 35 μM dCTP; 3.5 μM dGTP; 35 μM dTTP; 200 μM ddGTP
ddT: 35 μM cCTP; 35 μM dGTP; 3.5 μM dTTP; 200 μM ddTTP

All reactions were performed in glass capillaries in a final volume of 5 μl containing 0.2 units of DNA polymerase (Klenow fragment). After incubation for 15 min at room temperature, 1 μl of 0.5 mM dATP was added to each reaction and incubation was continued for 5 min at room temperature. If restriction endonuclease cleavage was necessary, 0.2 μl of restriction endonuclease was added to each reaction and incubated at 37 °C for 5 min. 10 μl of dye mix (90% deionised formamide, 0.25% bromphenol blue, 0.25% xylene xyanol) was added and the reactions were boiled for 3 min prior to gel electrophoresis. If restriction endonuclease cleavage was not necessary, the dye mix was added immediately following the dATP chase step. *Panel A*, pDV803 was prepared as a template by cleavage with *Pst*I followed by treatment with exonuclease III as described above. The primer was a 14-base pair DNA fragment isolated from a multiple digestion (*Hae*III + *Hpa*II + *Alu*I) of the 616-base pair fragment of Tn*9* (isolated from pDV803). The products of this reaction were fractionated on a 40 cm × 20 cm × 1.5 mm 12% polyacrylamide gel containing 25% glycerol in TB buffer. After electrophoresis, the gel was stained with ethidium bromide; individual bands were cut out and the DNA eluted from the acrylamide by soaking in 1.2 ml of buffer (0.3 M NaOAc, 0.1 M Tris-HCl, *p*H 8.0, 2.5 mM EDTA) for 16 h at 37 °C. The DNA fragments were then precipitated by addition of 2.5 volumes of ethanol, centrifuged in a SW50.1 for 30 min at 30,000 r.p.m., resuspended in distilled water and stored frozen at −20 °C. Primer–template hybridisation and chain elongation were as described above. There was no cleavage step with restriction endonuclease after extension with DNA polymerase (Klenow fragment). Sequence begins at nucleotide 73 and proceeds to the left (complement to the strand shown in Fig. 6). *Panel B*, 1.0 pmol of a 420-base pair DNA fragment generated by digestion with endonuclease *Hae*III was incubated in a volume of 15 μl containing 4 units of exonuclease III in 50 mM Tris-HCl, *p*H 8.0, 1 mM DTT, 1 mM $MgCl_2$ for 1 h at 37 °C. The mixture was then sealed in a capillary and boiled for 3 min. 5 μl of this mixture (0.33 pmol) was annealed with 0.1 pmol of M13pCmA DNA and used as a substrate for the primed synthesis reaction as described above. Electrophoresis in both panels was for 2 h at 35 W constant power on an 8% polyacrylamide–7 M urea gel (40 cm × 20 cm × 0.45 mm) as described by Sanger and Coulson[41]. The sequence begins at nucleotide 1,070 and proceeds to the left (complement to the strand shown in Fig. 6).

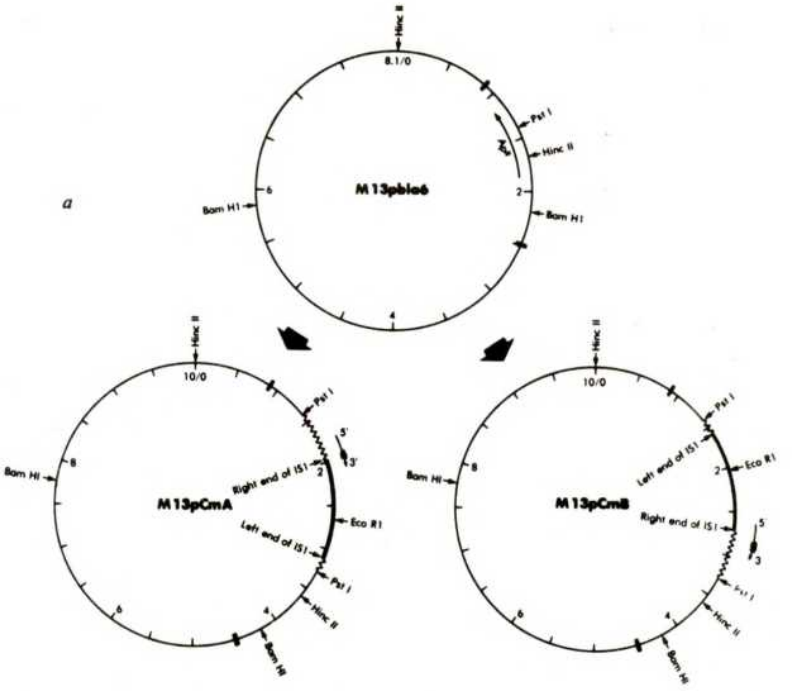
involved in the interaction with CAP–cyclic AMP complex. The CAP sites of the *lac*, *gal* and *araBAD* promoters are bounded by AT-rich sequences. Similarly, the putative CAP site in the CAT promoter is bounded by direct repeats of the AT-rich sequence GAATAAATA (Fig. 6, position 17–25 and position 50–58). Whether these sequences are also involved in the CAP–cyclic AMP interaction is not known.

Other features common to promoters are sites of the molecular interaction with RNA polymerase. Two common sites have been defined from mutational and sequence analysis of a number of different promoters. One of these is the heptanucleotide TATPuATPu (Pribnow box) which is centred approximately 10 nucleotides before the start point of transcription[29]. The other is the so-called −35 region which is centred approximately 32 nucleotides before the start point of transcription[30]. Although several such sequences are present in the CAT promoter, assignment of a specific role to these sequences awaits determination of the 5′ termini of the CAT mRNA(s).

The *ochre* codon at nucleotide 881–883, which is presumably the translational stop for the CAT gene, is immediately followed by the sequence TTTTTTTAA and preceded by a GC-rich region (13 of 14 base pairs). This region may represent the CAT mRNA termination site. This assumption is based by comparison with other genes in which similar sequences have been observed at the transcriptional termination site (for review, see ref. 31). However, the inverted repeat sequences found in these other instances (characteristic of ρ-dependent termination) which can potentially form stem-loop structures, are not found in the CAT DNA sequence.

Of the 1,102 base-pair region of Tn*9* bounded by the IS*1* sequences, at least nucleotides 28–883 are involved in the expression of CAT. An analysis of the sequence distal to the CAT translational stop signal reveals an uninterrupted coding sequence for 75 amino acids beginning with an AUG initiator codon at nucleotide 965 and extending 81 nucleotides into IS*1*. No other protein larger than 40 amino acids can be predicted from translation of all three reading frames of either strand of the 1,102 base-pair region.

Arber *et al.* have shown that Tn*9* carries fusidic acid resistance as well as chloramphenicol resistance[32]. Based on the sequencing data, fusidic acid resistance must be due to either



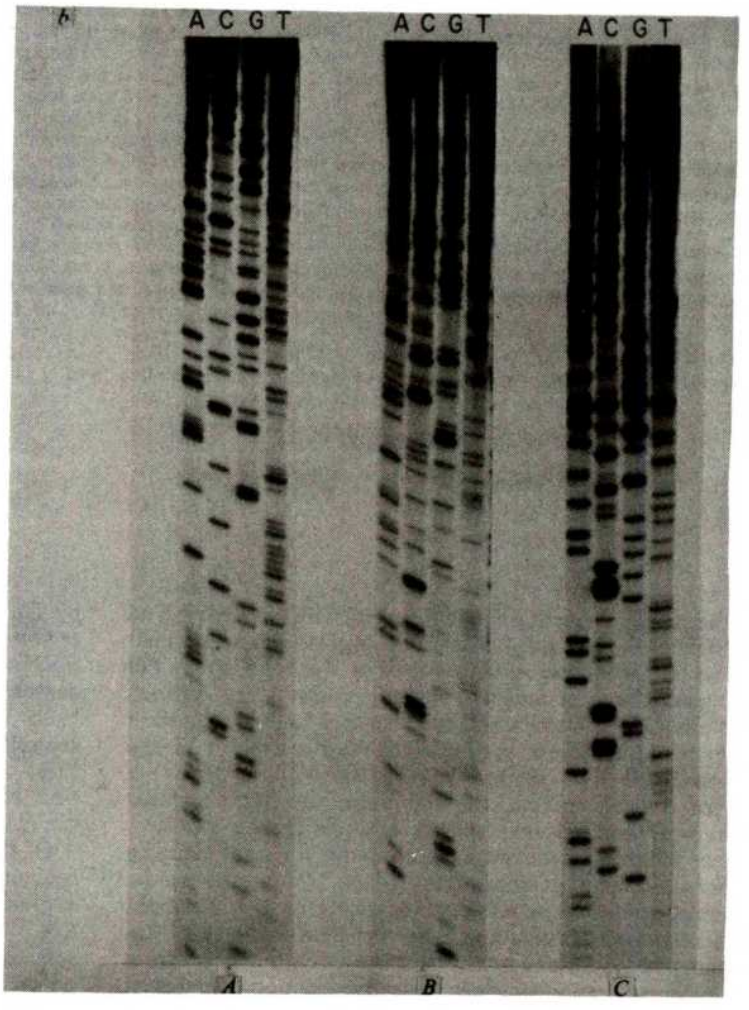


Fig. 5 *a*, Schematic representation of Tn*9* fragments cloned in M13pbla6. A derivative of M13 Tn3–15 was used as a cloning vehicle[13]. This phage, M13pbla6, contains the β-lactamase gene from Tn*3* inserted in the intercistronic region between genes II and IV of M13 (D. Ray and S. Hines, personal communication). Strains which harbour M13pbla6 are therefore ampicillin resistant. The single *Pst*I site in M13pbla6 was used to clone the 1,870 base-pair fragment of Tn*9* generated by digestion with *Pst*I. Since the *Pst*I site of M13 is within the β-lacatmase gene, insertion of the 1,870 base-pair fragment at this site resulted in phages which conferred the phenotype chloramphenicol resistance-ampicillin sensitivity on infected cells. RF DNA was isolated from infected cells with this phenotype as described below and analysed by restriction endonuclease digestion. Of 28 independent isolates screened, 26 carried the inserted fragment in one orientation (M13pCmA) and only two were in the other orientation (M13pCmB). Barnes[14] also noted an orientation effect in cloning the *His* operon DNA into M13. In that instance a slower growth rate and a lower yield of phage was observed when DNA was inserted in one particular orientation. Phage DNA was isolated from M13pCmA and M13pCmB and used as templates with primers prepared from restriction endonuclease generated fragments of pDV801, pDV802, and pDV803. Since the template is completely single-stranded, no template related problems were encountered when the primers were recleaved from the extended chains with restriction endonucleases. However, successful cleavage with restriction endonucleases was dependent upon the particular lot of endonuclease used. M13pbla6 was purified from infected cells as described by Messing[10]. 0.5 μg of this DNA was cleaved with *Pst*I and mixed with 0.35 μg of the 1,870 base-pair DNA fragment of Tn*9* (purified from pDV801). Ligation with T4 polynucleotide ligase was performed in a final volume of 50 μl of H buffer (see legend to Fig. 2) plus 0.4 mm ATP for 16 h at 5 °C. 0.5 μl of this mixture was used to transfect 0.1 ml of Ca^{2+} treated *E. coli* K802F'lac (ref. 10). The transfected cells were mixed with 0.2 ml of uninfected cells (4×10^7 cells) and overlayed on YT plates. Infected cells from individual plaques were tested for chloramphenicol resistance. Small cultures were grown from chloramphenicol-resistant infected cells and RF DNA was isolated as previously described for plasmid screens[37]. The RF DNA was digested with *Pst*I to determine whether the Tn*9* fragment was present, and with *Eco*RI plus *Hin*dII to determine the orientation of the inserted fragment. The two orientations (M13pCmA and M13pCmB) are shown schematically. The numbers indicate the size of the phage DNA in kilobases. To prepare M13pCmA and M13pCmB single-stranded DNA, the supernatant from a litre culture of infected cells grown in YT medium was adjusted to 0.5 M NaCl and the phage precipitated by the addition of polyethylene glycol to a final concentration of 4%. Precipitated phage were collected by centrifugation and resuspended in 10 mM Tris-HCl, *p*H 7.4, 10 mM $MgCl_2$. After a low speed spin (10,000 r.p.m. for 10 min) to remove cellular debris, the phage was lysed by heating in 0.5% SDS, 50 mM EDTA for 10 min at 65 °C. The phage DNA was extracted with phenol followed by extraction with phenol–chloroform and precipitated with 2.5 volumes of 95% ethanol. The DNA was further purified by centrifugation for 3 h in a 5–20% neutral sucrose gradient as described in the legend to Fig. 4. For use as a template, 0.1 pmol of DNA was used and the reactions carried out as described in the Fig. 4 legend. The arrows indicate the direction of chain extension after annealing with primers. *Fig. 5b*, Autoradiogram of a sequencing gel utilising M13pCmA or M13pCmB templates. pDV802 or pDV803 (6 pmol) were cleaved with *Pst*I plus *Eco*RI in H buffer containing 50 mM NaCl. The DNA was precipitated with 2.5 vol ethanol and resuspended in 30 μl of distilled water. 2.5 μl (0.5 pmol) of this DNA was mixed with 0.1 pmol of template (M13pCmA or M13pCmB) prepared as described above. After boiling for 3 min in a sealed capillary, annealing and chain elongation were carried out as described in the Fig. 4 legend. After incubation with *Pol*I and a 5 min chase with 1 μl of 0.5 mm dATP, 0.2 μl (10 units) of *Eco*RI (panels *A* and *B*) was added and the incubation continued at 37 °C for 5 min before addition of the dye mix. Electrophoresis on a 8% polyacrylamide–7 M urea gel was as described in the legend to Fig. 4. *Panel A*, M13pCmA template, pDV802 cleaved with endonucleases *Pst*I plus *Eco*RI as primer. Sequence begins at nucleotide 412 and proceeds to left (complement to strand shown, Fig. 6). *Panel B*, M13pCmB template, pDV803 cleaved with endonuclease *Pst*I plus *Eco*RI as primer. Sequence begins at nucleotide 470 and proceeds to the right (strand shown in Fig. 6). *Panel C*, M13pCmB template, 105-base pair *Alu*I generated DNA fragment primer. After extension and chase, 0.2 μl *Pvu*II was added to release the extended chains. Reaction conditions and primer purification were as described in the legend to Fig. 4. Sequence begins at nucleotide 373 and proceeds to the right (strand shown in Fig. 6).

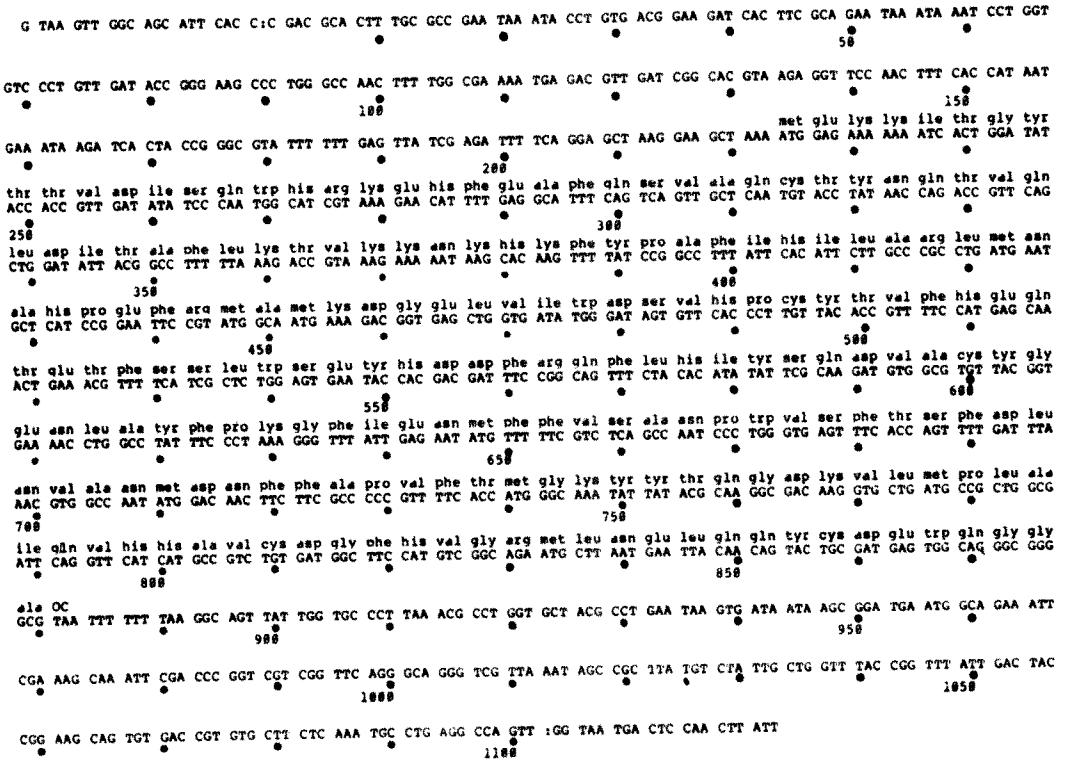


Fig. 6 Nucleotide sequence of the 1,102 base-pair region of Tn*9* between the direct repeats of IS*1*. The amino acid sequence of chloramphenicol acetyl transferase predicted from the nucleotide sequence is indicated above the respective codons. Numbering of nucleotides beginning at the left end of IS*1* (designation of Ohtsubo and Ohtsubo, ref. 15) is as shown. The junctions with IS*1* are indicated by a colon (:). 20 nucleotides from both the left and right ends of the IS*1* sequences present in Tn*9* are also shown.

one of the small putative peptides coded for by this region or by the CAT gene itself. If the CAT gene is involved in fusidic acid resistance, the biochemical basis for such an effect is obscure[33].

The complete nucleotide sequence of a transposon encoding a heat stable toxin from *E. coli* (Tn*1681*) has recently been completed (M. So, F. Heffron and B. McCarthy, personal communication). Although the IS*1* sequences are directly repeated in Tn*9*, this arrangement does not seem to be required for transposition as Tn*1681* is bounded by inverted repeats of IS*1* (ref. 34). A comparison of the internal sequences of Tn*9* and Tn*1681* bounded by the IS*1* sequences reveals no significant homology. Furthermore, there is no apparent relationship between the sequence at both internal IS*1* junctions in either Tn*9* or Tn*1681*. Although the putative protein described above could be involved in transposition, we feel this is unlikely since IS*1* itself is transposable and, in the case of Tn*1681*, no protein initiating within the internal sequences of Tn*1681* and extending into IS*1* can be predicted from the nucleotide sequences. Taken together these data suggest that in transposons bounded by insertion sequences, like Tn*9* and Tn*1681*, the information required for transposition resides within the flanking insertion sequences. In contrast, the internal regions of transposons like Tn*3* (encoding ampicillin resistance), which are not flanked by insertion sequences encode functions necessary for their own transposition[35]. In either case, a contribution of host functions to the transposition process is not ruled out.

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Primary structure of a chloramphenicol acetyltransferase specified by R plasmids

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Naturally occurring isolates of chloramphenicol-resistant bacteria commonly synthesise chloramphenicol acetyltransferase (EC 2.3.28; CAT) in amounts which are sufficient to account for the resistance phenotype and often harbour plasmids which carry the structural gene for CAT[1,2]. The finding of CAT in such diverse prokaryotes as *Proteus mirabilis*, *Agrobacterium tumefaciens*, *Streptomyces* sp., and a soil *Flavobacterium* has led to speculation concerning the origin and evolution of the more commonly observed CAT variants specified by plasmids in clinically important bacteria[2]. To provide a more solid basis for studying the evolution and spread of CAT within prokaryotes we chose to determine the complete amino acid sequence of a type I variant of CAT, the variant known to be associated with most F-like plasmids conferring chloramphenicol resistance. The sequence has been determined by combining the results obtained from manual and automated sequential degradation with those obtained by mass spectrometry of peptides generated by enzymatic digestion. The directly determined primary structure is identical with that predicted by the DNA sequence analysis[3] of the chloramphenicol resistance transponson Tn*9* known to specify a type I variant of chloramphenicol acetyltransferase.

CAT catalyses the acetylation of chloramphenicol to yield as the inital product 3-acetoxychloramphenicol which is devoid of significant antibiotic activity[4]:

chloramphenicol + acetyl-S-CoA → 3-acetoxychloramphenicol + HS-CoA

The plasmid-borne chloramphenicol resistance conferred by CAT in *Escherichia coli* and other related Gram-negative bacteria is a constitutive property of such strains whereas staphylococci synthesise CAT only after exposure to the antibiotic or closely related compounds including a gratuitous inducer which neither serves as a substrate for CAT nor is an antibiotic[5]. Three reasonably well defined and divergent types of CAT have been shown to be mediated by plasmids in Gram-negative bacteria whereas staphylococcal plasmids appear to specify at least four closely related variants[6,7].

All CAT variants studied to date appear to exist in solution as tetramers of four identical subunits[8]. The CAT monomers of all variants possess molecular weights in the range 22,000–24,000 by SDS-polyacrylamide gel electrophoresis. In the light of the calculated molecular weight for the complete 219 amino acid primary structure of the enzyme described below, it seems likely that the expected monomeric molecular weights of most if not all CAT variants will need to be revised upwards.

The yield of pure protein was maximised without recourse to recombinant technology by taking advantage of several intrinsic advantages of the system. High level chloramphenicol resistance mutants can be isolated by selection for other plasmid-linked drug resistance markers, yielding high copy number plasmids[9]. Furthermore, the expression of CAT is known to be subject to cyclic AMP-mediated catabolite repression and, hence, growth of bacterial cells on glycerol rather than glucose can lead to up to fivefold increases in yield of CAT[10]. Finally, the development of a reproducible high capacity affinity chromatographic method for the purification of CAT has given almost quantitative recovery of electrophoretically homogeneous material in a single purification step[11]. One gramme of pure CAT specified by plasmid JR66b was used for most of the studies described. Supplementary information has been provided by structural studies on smaller amounts of a closely related type I variant specified by plasmid R429 (ref. 12).

The initial approach to determining the complete covalent structure of CAT took account of several preliminary observations. The protein was known to contain not less than seven methionines, one of which was N-termional and unblocked. Although tryptic digests of the reduced and carboxymethylated protein yielded useful peptide maps, there were regions of structure not likely to be represented in soluble peptides. By contrast and as expected, digests of CAT with chymotrypsin and elastase went smoothly. The overall plan was therefore as follows: (1) determine the N-terminal amino acid sequence by automated Edman degradation; (2) prepare and sequence fragments isolated after cleavage at methionines with cynaogen bromide, using both automated Edman degradation and enzymic sub-digestion followed by manual 'dansyl' Edman methods; and (3) explore the use of mass spectrometry (MS) to determine the amino acid sequence of fragments generated by chymotrypsin, elastase and subtilisin. A particular advantage of the use of mass spectrometry was not only the relative speed with which the sequence of many small (three to nine residue peptides can be determined but also the attraction of 'mixture analysis' to get sequence information from column or paper fractions containing several peptides[13]. In addition to these three main approaches we also took advantage of the results obtained by dansyl Edman and automated sequence analysis of certain tryptic peptides and results of manual sequencing of chymotryptic or elastase peptides to extend the information from the mass spectrometry results. The techniques used have been described in detail elsewhere[13–18].

The complete amino acid sequence of CAT is shown in Fig. 1. The data have been presented in a manner which, although unconventional, may help to show (1) the amount of redundant information insuring accuracy throughout most of the structure, (2) the regions where overlaps or redundancy are limited, and (3) the very large fraction (182 residues or 83%) of the structure which was determined by mass spectrometry. In addition, it may be useful to note that the heavy dark line above the sequence indicates the structure also determined unambiguously for the type I CAT specified by plasmid R429. The sole differences detected between these two proteins thus far have been the tighter binding to affinity resins of the enzyme encoded by R429 and the amino acid difference at residue 162, the substitution of Ala for Asn.

Several regions of the structure were particularly troublesome. The N-terminal sequence analysis by Edman degradation using both liquid ('spinning cup') and solid phase techniques gave a sharp drop in signals after Gln 15, probably due to cyclisation and blockage. The crucial chymotryptic peptide spanning the region from His 17 to Phe 25 was in low yield and contained Arg. Although sequenced by the dansyl-Edman approach, it was only correctly placed in the sequence when it became obvious that it was contained in the C-terminal part of the elastase fragment spanning Asp 12 to Ser 27. The 'expected' L4 tryptic peptide (Glu 20 at N-terminus) was never isolated and CB-2 was a low-yield 66-residue fragment which was known only by its analysis, and by N- and C-terminal sequences.

A second region of the structure which gave rise to difficulties until the late stages of the project was the two amino acid overlap at 162–163, determined both by dansyl-Edman and mass spectrometry on the elastase fragment Asn 162 to Val 170.

Our search for ambiguities and errors in overlapping peptides in the regions mentioned was prompted by access to the DNA sequence data of the Basle group[26] for the region of their transposon which corresponded to the first 142 amino acids of

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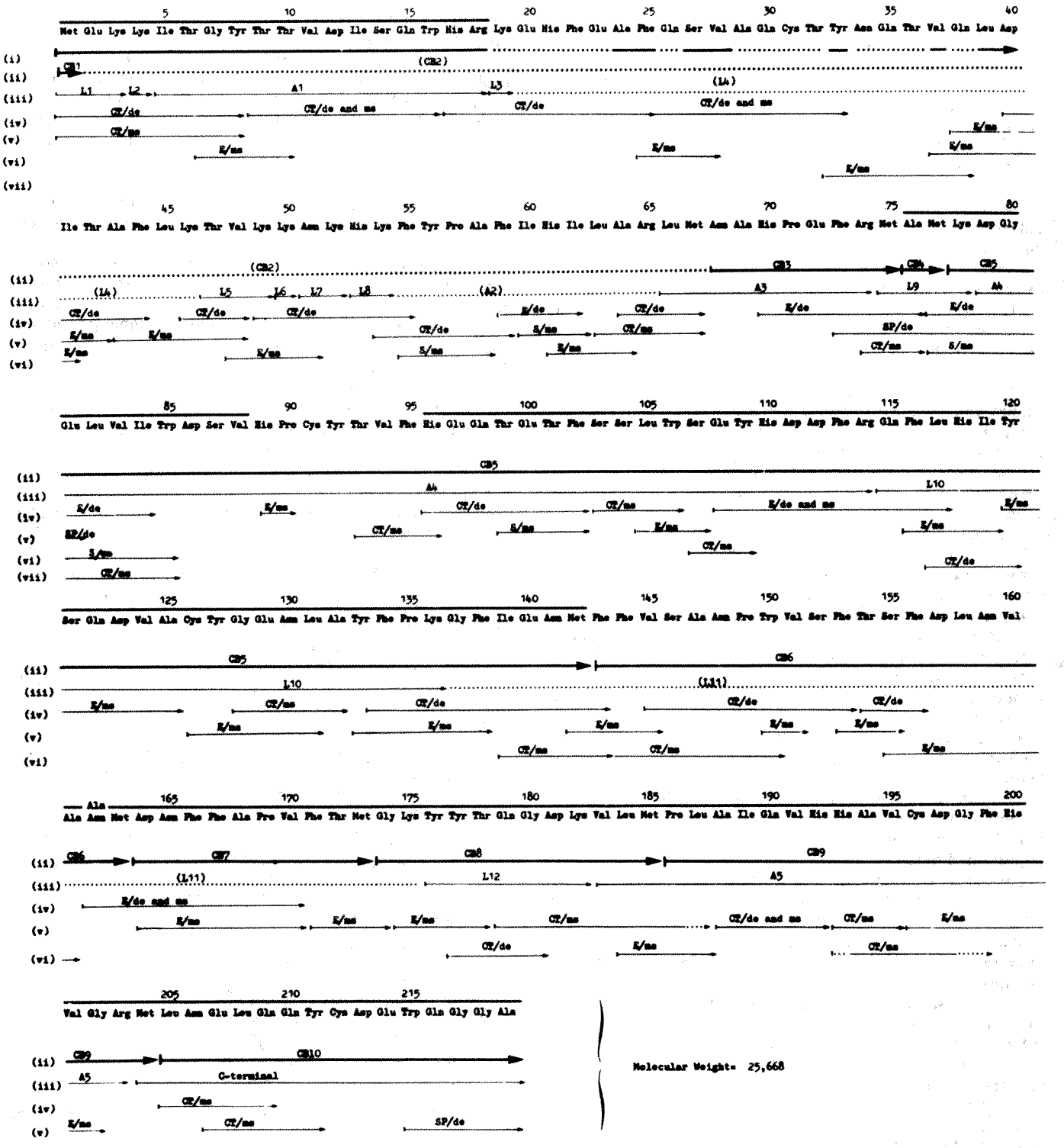


Fig. 1 Amino acid sequence of chloramphenicol acetyltransferase specified by plasmid JR66b. Line (i) indicates the results of automated Edman degradation to residue 40. Dotted underlining indicates ambiguous results or no signal on thin layer, gas chromatography, or back hydrolysis of PTH amino acids. Line (ii) shows cyanogen bromide fragments which were sequenced either by automated Edman degradation or subdigestion with proteases to yield smaller peptides which were sequenced by the dansyl-Edman technique. Line (iii) shows the tryptic fragments and lines (iv–vii) indicate peptides generated by digestion of the protein with chymotrypsin (CT), subtilisin (S), elastase (E), or staphylococcal protease (SP) and sequenced by mass spectrometry (ms) or the dansyl-Edman method (de). All cyanogen bromide digests were performed on *S*-cysteinyl[^{14}C]carboxymethylated protein and the fragments were purified by a combination of gel filtration in 20% formic acid or 6M guanidine hydrochloride and DEAE-cellulose chromatography. One chymotryptic digest was performed on performic acid oxidised protein and one on enzyme which had been *S*-aminoethylated with ethylene imine. The elastase and staphylococcal protease digests were performed on ^{14}C-carboxymethylated protein. Peptides from chymotrypsin and elastase digests were purified by Dowex 50 ion-exchange chromatography using a pyridine-acetate eluant or by DEAE-cellulose chromatography with a linear gradient of ammonium bicarbonate. High voltage paper electrophoresis and chromatography were used for purification of pooled column fractions after removal of salt by lyophilisation or gel filtration on Sephadex G-10. The numbering system for tryptic peptides takes into account hypothetical peptides which were never isolated (dotted lines). The same convention was used for cyanogen bromide fragments. The heavy line above the amino acid sequence indicates the extent of sequence analysis performed on the CAT specified by plasmid R429, all of which is identical with that of plasmid JR66b save for Asn 162 which is Ala in the R429 variant of CAT.

CAT. Re-alignment of the peptides in the regions noted above yielded the primary structure shown in Fig. 1.

Although the data summarised in Fig. 1 are presented in such a way as to summarise the evidence from which the structure has been deduced, we have drawn attention to regions which may be of functional importance. In the native enzyme the chloramphenicol binding site seems likely to include portions of the structure near Cys 31 as well as the segment from His 192 to Cys 196 (ref. 19). Studies done on a staphylococcal variant of CAT, which shows a high degree of conservation in the latter region but less than 50% homology elsewhere, have implicated His 192 on the basis of experiments with alkylation by iodoacetamide and diethylpyrocarbonate inhibition[17]. The protection afforded by chloramphenicol to inhibition at all three residues is not seen with acetyl-S-CoA. In the fully folded native structure, the coenzyme-binding domain may exist within the N-terminal one-third of the polypeptide. The evidence on this point is both circumstantial and indirect; the truth will be known with certainty on the completion of high resolution X-ray diffraction studies which are in progress[20]. However, we note that there is a potential adenine nucleotide binding site in the N-terminal one-third of the polypeptide where application of the secondary structure parameters of Chou and Fasman[10] yields a desired domain consisting of alternating β structure and α-helix[22].

The matter of the points of contact between subunits of the CAT tetramer has recently been approached in the light of earlier studies on the ease with which very stable hybrid CAT tetramers can be generated *in vivo* and *in vitro*[12]. It seemed likely that salt bridges between monomers might be involved and that a search for 'buried' lysine residues might reveal one or more which, especially if found consistently among CAT variants, might be prime candidates for sites of contact between subunit interfaces. The strategy used was an extension of that described previously[23] and involved treatment of the native tetramer with nonradioactive methyl acetimidate followed by removal of the reagent, unfolding of the protein in guanidine hydrochloride and treatment with ^{14}C-labelled methylacetimidate to label any lysine residue previously shielded from solvent and unlabeled reagent. A unique amino group was modified by the radioactive imidoester, that of Lys 136 (L.C. P., unpublished data).

The amino acid sequences of most proteins—in the absence of further information on secondary or tertiary structures or collateral data on related proteins—are not particularly provocative, and the structure for CAT is no exception. Although much of the primary structures of two other plasmid coded CAT variants are known (*E. coli* type III and *Staphylococcus aureus* type C), an assessment of homology would be premature, apart from that which has already been made concerning the putative chloramphenicol binding site. Similarly, X-ray diffraction studies have not yet proceeded to a stage where useful inferences may be drawn.

The completion of the nucleotide sequence of Tn*9* by Alton and Vapnek[3] and by the Basle group[26] and the rapid progress made by other groups on analogous or homologous chloramphenicol resistance transposons have reinforced the points made by Sutcliffe that DNA sequence analysis offers compelling advantages in terms of time and resources as compared with protein sequencing[24]. On the other hand, the availability of limited amino acid sequence data on a structural gene of interest may be of considerable assistance as a guide to ambiguities in reading frame and is essential for anticipating post-translational modifications[25]. The evidence presented in this communication, that of the companion paper[3] and that of Marcoli *et al.*[26] indicate that in the case of the type I variant of CAT the results of the two approaches give identical results. Further studies on the CAT 'family' of proteins are now likely to proceed more rapidly as nucleotide sequence analysis replaces amino acid sequencing as the primary tool for generating primary structures for evolutionary studies and for the interpretation of electron density maps. However, we wish to stress the continued importance and necessity of protein chemistry for studies of catalytic function, subunit interactions, and ligand binding.

This work was initiated at the MRC Laboratory of Molecular Biology, Cambridge where W.V.S. and B.D.B. were visiting scientists. Much of the mass spectrometry work was done in the University Chemical Laboratory, Cambridge. We are grateful to both institutions for assistance and support. John Bridgen and John Walker were especially helpful with automated N-terminal sequence analysis. Subsequent studies were supported by the MRC in the form of a project grant (Leicester) and a programme grant (Imperial College). We thank F. Bolivar for base sequence data for the regions flanking the *Eco*RI site (Glu 72/Phe 73), information which first alerted us to an overlap error. We are particularly grateful to T. Bickle, S. Iida and R. Marcoli for early discussions of their proposed structure for the transposon derived from plasmid NR1, and we thank N. K. Alton and D. Vapnek for sharing their data before submission for publication.

Note added in proof: The primary structure of a transposable element smaller and analogous to that specifying the type I variant of CAT has now been determined[26]. Knowledge of the amino acid sequence of CAT and the nucleotide sequence of Tn*9* is likely to be particularly useful in the light of recent independent experiments by two groups which show that the gene product of Tn*9* is expressed well in yeast (*Saccharomyces cerevisiae*) when the chloramphenicol resistance determinant is part of a cointegrate composed of the yeast 2-μm plasmid and an *E. coli* vector (J. D. Cohen, R. Eccleshall, R. D. Needleman, H. Federoff, B. A. Buchferer and J. Marmur; and C. Lichtenstein and C. Scazzocchio; personal communications to W.V.S.).

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E. coli ribosomal RNA contains sequences homologous to insertion sequences IS*1* and IS*2*

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The insertion sequence (IS) elements, IS*1* and IS*2*, present in multiple copies in the *Escherichia coli* chromosome[1–3], are transposable genetic elements of known nucleotide sequence[4,5]. These elements can modulate gene expression[6–7], but it is not known whether they normally function in genetic control. To

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determine whether IS elements could exert control through specific RNA transcripts, we hybridised λNNC$_{1857}$r14 (carrying IS*1*) and pBR322 (carrying a portion of IS*2*) to Northern blots[8] of *E. coli* RNA. Regions of homology between the IS elements and ribosomal RNA were observed. Computer analysis of reported nucleotide sequences detected large segments of homology between the IS elements and both 23S and 16S rRNA. Additional homologous sequences in ΦX174 and a leader region of a ribosomal protein gene cluster were also detected. The homologous sequence between IS*2* and 16S rRNA is the same sequence in ΦX174 DNA which codes for the ends of the *E* and *D* gene and the start of *J*. The partial IS sequences may represent silent evolutionary remnants or they could modulate the expression of genes carrying these sequences.

IS elements are specific segments of DNA which can undergo *rec*A-independent translocation between DNA sequences with no apparent homology. These elements can modulate gene expression, cause polar mutations when integrated into proximal regions of operons, and act as vehicles for chromosomal rearrangements[6,7]. They inactivate genes into which they insert, and reactivate them on precise excision. Two insertion sequence elements, IS*1* and IS*2*, which are present in multiple copies in the *E. coli* chromosome[1-3], have been entirely sequenced[4,5]. IS*1* contains several translation stop signals in all three reading frames and causes polar mutations when inserted in either orientation. Integration of IS*2* in one orientation can cause initiation of transcription of a proximal gene presumably because the IS sequence contains a promoter[10]. Integration of IS*2* in the opposite orientation causes transcription termination, possibly due to the presence of a *rho*-sensitive transcription termination signal[11]. IS*2* also contains several translational stop signals in all three reading frames[5].

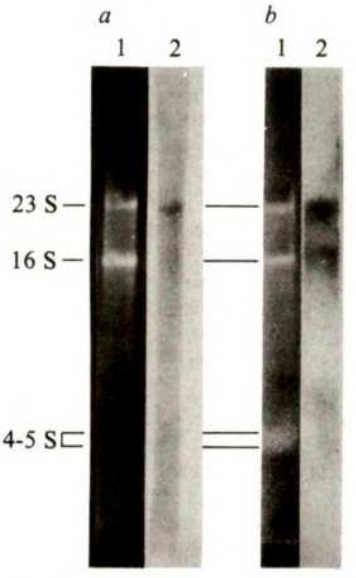


Fig. 1 Hybridisation of IS*1* and IS*2* to *E. coli* RNA. Whole cell RNA from an RNase$^-$ strain of *E. coli* K12 (Miles) was treated for 30 min at 37°C with 10 μg ml^{-1} of RNase-free DNase (Worthington) and then inactivated by heating to 65°C for 3 min. The RNA was then separated into its component size classes by 2% agarose gel electrophoresis in 20 mM phosphate buffer, *p*H 7.5. Ethidium bromide visualisation of two separate experiments are seen in *a*1 and *b*1. The RNA in the gels was blotted and bound to treated filter paper as described elsewhere[4]. Wild-type λ and λNNC$_{1857}$r14 (IS*1*) DNA obtained from purified phage particles[5], and purified plasmid pBR322 and pBR322 (IS*2*) DNA (described in ref. 9) were nick-translated by the method of Rigby *et al.*[20] using ^{32}P-dCTP (>300 Ci mmol^{-1}, NEN). These probes were hybridised to the above RNA containing filters as described[9] with the following exceptions: tRNA was used as cold carrier, and the filter paper was washed after hybridisation four times in hybridisation buffer at 37°C for 30 min, then four more times in 0.2 × SSC, 0.1% SDS at 37°C for 30 min. Wild-type λ DNA and pBR322 (without the IS*2* insert) did not hybridise to the filters. Autoradiograms of hybridisation of λNNC$_{1857}$r14 (IS*1*) and pBR322 (IS*2*) are seen in 1*b* and 2*b*, respectively. The predominant 23S, 16S and 4–5S rRNA size components are indicated.

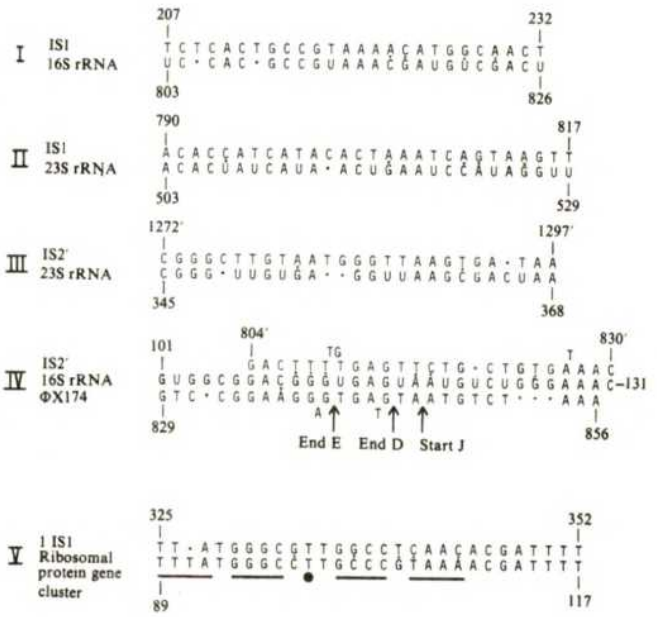


Fig. 2 Nucleotide sequence homologies detected by computer analysis. The computer program described by Korn and coworkers[13], as modified by Friefeld and Brutlag, was used to compare the nucleotide sequences of IS*1* (ref. 4) and IS*2* (ref. 5) to a portion of 23S rRNA, 16S rRNA[12], ΦX174 DNA[17], and a 3,000-base pair ribosomal protein gene cluster[18]. The numbers correspond to the coordinates from the published sequence. Primes correspond to the complementary strand of the reported sequence in inverted order. Dots indicate mismatches. The sequences shown are all considered statistically significant by the criteria and calculations defined by Brutlag.

There has been speculation concerning the role of IS elements in gene expression and evolution[6,7], but it is not clear whether these elements normally function in genetic control. Further, it is not known whether these elements act only at the DNA level (that is, promoter sequences, sequences inducing polarity), or can control gene expression at the post-transcriptional level. If portions of the IS elements exert their function through specific transcripts, then regions of homology between the IS elements and *E. coli* RNA should be detectable. To examine this possibility, labelled probes containing IS*1* and IS*2* were hybridised to Northern blots[8] containing total *E. coli* RNA which had been fractionated by electrophoresis on agarose gels. In a previous study, regions of limited homology between IS*1* and IS*2*, not ordinarily observable by solution hybridisation techniques, were readily detected by hybridisation to DNA restriction fragments blotted to nitrocellulose filters[9]. In the present study, total *E. coli* RNA was separated into its predominant 23S, 16S and 4–5S size components by agarose gel electrophoresis (Fig. 1, lanes *a*1, *b*1) and chemically bound to filter paper as described by Alwine and coworkers[8]. ^{32}P-labelled DNA from wild-type bacteriophage λ, λNNC$_{1857}$r14 (containing IS*1*), plasmid pBR322, and pBR322 which contains two-thirds of IS*2* plus a small segment of λ DNA was then hybridised to strips of filter paper containing the separated RNA.

The autoradiograms in Fig. 1, lanes *a*2, and *b*2, show the result of the hybridisations of λNNC$_{1857}$r14 (IS*1*) and pBR322 (IS*2*), respectively, to *E. coli* RNA. IS*1* hybridised pre-

dominantly to 23S rRNA and to a lesser extent to 16S rRNA. IS*2*, however, hybridised equally well to both 23S and 16S rRNA. Due to the exceedingly short half life and unique complexity of mRNA, this experimental approach would probably not detect similar sequence homologies with the less abundant messenger RNA fractions which are present on the filter between 16S and 4–5S rRNA. λ and pBR322 probes without the IS elements, isolated in an identical manner to those containing inserts, did not hybridise to the RNA. Furthermore, the purified RNA was treated with DNase before electrophoresis to exclude the possibility of chromosomal DNA contamination.

A large portion of the sequence of the 16S and 23S rRNAs from *E. coli* is known (ref. 12 and C. Squires, personal communication). Therefore, with the aid of a computer program devised by Korn and coworkers[13] we attempted to find the regions of homology by comparing the nucleotide sequences of the IS elements with that of the RNAs. Sequences homologous to large regions of IS*1* and IS*2* (greater than 25 base pairs) were found in both 23S and 16S rRNA (Fig. 2). In addition to the sequences shown, numerous shorter regions of homology were detected between the IS elements and the 23S and 16S rRNAs. These shorter sequences were found in clusters in regions which had extensive hyphenated homology. In the case of the 23S rRNA, the regions which can form secondary structure in the RNA (inverted, complementary repeats) involve the same sequences which have hyphenated homology with the IS elements. The regions of IS*1* and IS*2* found in 23S rRNA (Fig. 2II, III) correspond to the terminal regions of these IS elements.

The portions of the IS elements present in ribosomal RNA can be viewed as nucleotide sequences that have become stably integrated over the course of evolution. The IS elements, or sequences like them, could at one time have interrupted DNA sequences coding for ribosomal RNA; the sequences remaining in the rRNA could represent remnants left behind by imprecise excision or duplication. It is also possible that IS elements which interrupt ribosomal DNA genes are partly processed out at the RNA level.

The partial IS sequences detected in this study may represent regions that have been conserved because of a required role in ribosome function. For example, homologous regions between IS elements and ribosomal RNA could modulate the translation of mRNA carrying IS elements or portions of these elements. Messenger RNA carrying IS sequences could be recognised by the ribosomes by virtue of the sequence homology between the IS elements and 23S and 16S rRNA described here. Hybridisation between the putative IS-mRNA and the ribosomal region containing IS homology could alter an essential RNA–RNA or RNA–protein interaction within a region of extensive secondary structure in the ribosome, making the ribosome either more or less efficient. One consequence of this mechanism would be to protect the cell from insertions which would adversely affect essential functions. This type of mechanism, to circumvent gene inactivation due to IS insertion, predicts that portions of IS sequences should be found in mRNA. We therefore examined other known coding sequences for regions homologous to the insertion elements to determine if regions of partial IS homology resided in specific regulatory sequences. Significant regions of homology were found between IS*2* and ΦX174 DNA and IS*1* and a ribosomal protein gene cluster, as shown in Fig. 2.

To test whether homologous sequences similar to those shown in Fig. 2 for the IS elements, rRNA, ΦX174 DNA and the ribosomal protein gene cluster could be found between any large DNA or RNA, the nucleotide sequences of IS*1*, IS*2*, 23S rRNA, 16S rRNA, the plasmid pBR322 (ref. 14), SV40 DNA[15], and two histone genes[16] were compared with each other to identify regions of homology. The only significant regions of homology found, other than those shown in Fig. 2, were between IS*1* and IS*2* (ref. 9).

Homology between IS*2* and ΦX174[17] detected by computer analysis (Fig. 2, IV) was directly observed when labelled ΦX174 DNA was hybridised to a Southern blot containing IS*2* DNA. The region of the ΦX174 chromosome which is homologous to a 30-base pair nucleotide sequence in IS*2* DNA corresponds to the terminus of the overlapping *D* and *E* genes continuing through to the start of the *J* gene. As shown by Sanger and coworkers[17], this sequence contains a ribosome-binding site which is complementary to the 3′ end of 16S rRNA. Remarkably, the homologous sequence between ΦX174 DNA and IS*2* overlaps the region of homology between IS*2* and a region near the 5′ terminus of the 16S ribosomal RNA.

The 3,000-nucleotide sequence coding for a ribosomal protein gene cluster[18] includes the genes for ribosomal proteins L11, L1, L10, L7/L12, a portion of the gene for the β subunit of RNA polymerase, and two promoter regions (P_{L11} and P_β). A 95-base pair leader sequence before the first ribosomal protein (L11) structural gene contains a 23-base sequence which has dyad symmetry. A nucleotide sequence spanning the entire palindrome in this transcribed leader sequence was found to be homologous to a 29-base sequence in IS*1* (Fig. 2V). This homology is especially interesting in light of the observation made by Rak[10], who showed that *gal* RNA isolated from a mutant strain which contained an IS*2* insertion in the *gal* operon carried a short nucleotide sequence homologous to IS*2* DNA. The orientation of the insertion was such that it could act as a promoter of transcription. Because IS elements contain transcription and translation control sequences, it is possible that the type of homologous sequences found between IS*2* and the leader sequence in the ribosomal protein gene cluster represent recognition sites into which IS*1* and IS*2* can integrate and thus affect gene expression. This idea is supported by the fact that most of the IS*1* and IS*2* insertions into the *gal* operon occur in the leader sequence[19].

Several observations suggest a correlation between the potential for secondary structure in DNA and RNA and the regions containing sequences homologous to the IS elements: (1) the terminal regions of IS*1* and IS*2*, which are inverted repeats, have sequence homology with rRNA; (2) the sequence homology between IS*1* and the ribosomal protein gene cluster involves a palindrome in the leader region of the protein gene cluster; (3) the clusters of hyphenated homology between IS*1*, IS*2* and 23S rRNA correspond to the regions of putative secondary structure in the RNA. This correspondence between the location of IS sequences homologous to rRNA and the presence of secondary structure may have practical significance in the coordination of gene control throughout several levels of the regulatory hierarchy.

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X-ray crystallography of the binding of the bacterial cell wall trisaccharide NAM-NAG-NAM to lysozyme

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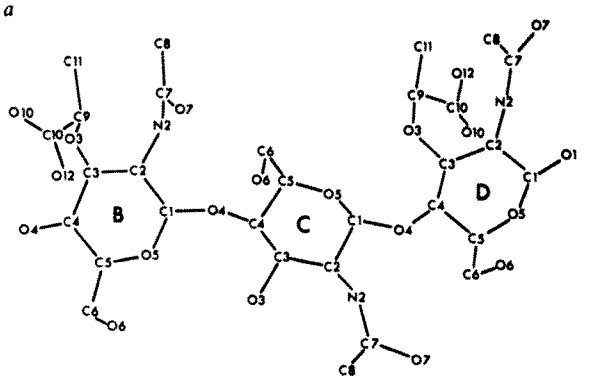
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Hen egg white lysozyme was the first enzyme whose structure was determined by X-ray crystallography[1]. The proposed mechanism[2-4] based on this structure involves the distortion of the saccharide residue (2-acetamido-2-deoxy-D-muramic acid, NAM) in the natural substrate[5] (an alternating β(1→4) linked oligomer of 2-acetamido-2-deoxy-D-glucose (NAG) and NAM residues) bound to site D in the binding cleft. The importance of substrate distortion has prompted numerous enzymatic[6], chemical[7], theoretical[8-12], and physical[13] studies, but there is little direct crystallographic evidence on the conformation of a NAM residue bound at site D. We now present the X-ray structure of the non-hydrolysed[13] trisaccharide NAM-NAG-NAM bound in subsites B, C, D. Our interpretation of the 2.5-Å resolution difference map does not involve distortion of this residue in site D. Comparison with the structure of the δ-lactone derived from tetra N-acetylchitotetraose ((NAG)$_3$NAL) bound to lysozyme[14] suggests we may be looking at a Michaelis complex.

Crystals of native hen egg white lysozyme (Sigma, lot L-6876) were grown by a vapour diffusion technique[15]. The crystals of lysozyme with the NAM-NAG-NAM(MGM) trisaccharide bound were grown by co-crystallisation from a solution of 8 mg of MGM and 15 mg lysozyme in 0.5 ml of 4% NaCl buffered with 0.1 M NaOAc at *p*H 4.5. The native and MGM-bound lysozyme crystals displayed remarkably different habits thus giving an early indication that MGM had been incorporated into the tetragonal crystals of lysozyme in spite of the relatively small binding constant, $3.6 \times 10^2\ M^{-1}$ (ref. 13). The unit cell dimensions of the native hen egg white lysozyme crystals are $a = b = 79.08$ (2) and $c = 38.02$ (1) Å, whereas those for the crystals of MGM bound lysozyme are 78.97 (2) and 38.25 (1) Å.

The structure factor amplitudes for both the native and the enzyme–trisaccharide complex were brought to the absolute scale using the scale factors determined from Wilson-type plots of the corrected intensity data[16]. The appropriately scaled data sets thus had an agreement factor of 0.120 ($\Sigma||F_{MGM}| - |F_N||/\Sigma|F_N|$) for 3,652 common reflections. The phases for the reflections of native lysozyme for the calculation of the difference map were those from the refined tetragonal structure which had a conventional R of 0.23 (refined phase angles supplied by Ms D. Grace). Initial examination of a difference mini-map contoured at intervals of 0.03 e Å^{-3} starting with ±0.10 e Å^{-3} confirmed binding of the substrate MGM to sites B, C and D of lysozyme. Detailed analysis of the map and fitting of the model to the electron density were carried out on an MMS-X interactive graphics system devised by David Barry of Washington University. The model of MGM used for fitting was constructed by docking the atomic coordinates of two crystal structures: β(1→4), α-*N*,*N*′-diacetylchitobiose (monohydrate) by Mo and Jensen[17] and *N*-acetyl-α-D-muramic acid by Knox and Murthy[18]. The atom numbering scheme for MGM is given in Fig. 1*a*.



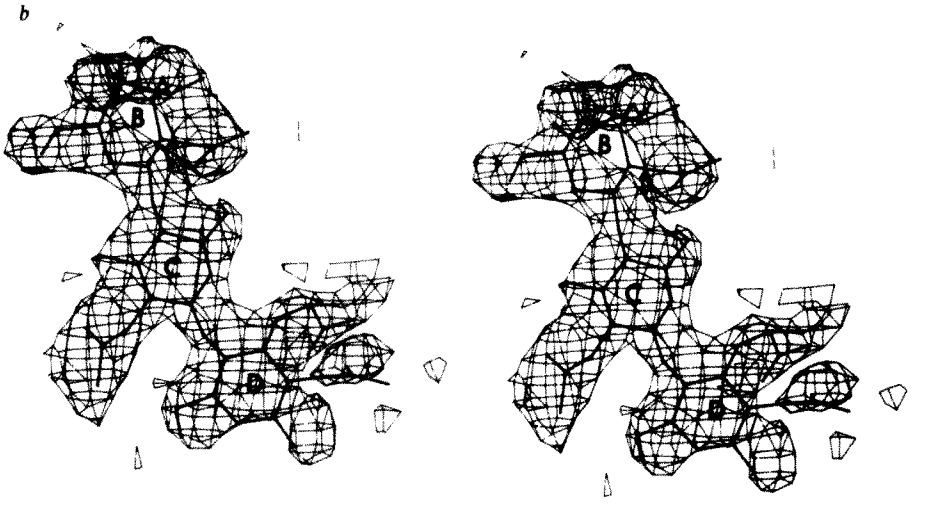


Fig. 1 *a*, The atomic numbering of the molecule of the trisaccharide, NAM-NAG-NAM, that was used in this study. Crystals of the NAM-NAG-NAM bound form of lysozyme were grown by co-crystallisation of MGM and lysozyme. Intensity data were collected from a crystal of native lysozyme and from a crystal of the MGM bound form on a Packer FACS-1 diffractometer[19]. Data from 10.0–2.5 Å resolution were measured from two symmetry equivalent (hkl and khl) reflections of each crystal. After correction of each data set for absorption[20] (maximum correction 1.31 for the native crystal; 1.51 for the MGM bound crystal), radiation damage[21] (maximum decay correction at $2\theta = 36°$ was 14% for native and 25% for the substrate bound crystal) and Lorentz-polarisation correction[22], the two symmetry equivalent reflections were averaged. The symmetry equivalent agreement indices for the intensity data thus measured are 2.86% for the MGM/lysozyme crystal and 2.30% for the native crystal. *b*, Stereoview of the difference electron density distribution ($|F_{MGM}| - |F_N|$), α_N with the fitted model of NAM-NAG-NAM superimposed. The sugar residues are labelled B, C and D to correspond to the binding sites on the lysozyme molecule[1-4]. This and subsequent figures were produced on an MMS-X interactive graphics system at the University of Alberta.

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The model was initially positioned in the electron density by making use of prominent features of the well-defined density associated with the ring in site C, that is, C_6–O_6 and the 2-acetamido group (Fig. 1*b*). In this and all subsequent manipulations of the model into the electron density, only allowed rotations about single bonds were used. Ring puckers were all left in the $^4C_1$ chair conformation for the final fitting procedure. Attempts were made to fit the alternative conformations for the sugar residue in site D, which were discussed by Ford *et al.*[14] into the electron density associated with the trisaccharide MGM. All conformations other than the $^4C_1$ chair for this residue fit less well. In these latter trials, atoms C_1 or O_1 were positioned too far out of the electron density contour surface.

Fitting of the lactyl moieties of both rings B and D was problematic. Simple rotations about permissible bonds produced no better fit for either group. The bond angles at O_3 and C_9 were increased to try to move the lactyl groups into the flattened density. However, no improvement of fit was achieved, so these groups were left with standard bond angles and lengths.

The difference electron density map in the regions of the gluco-pyranose rings in sites B and D is affected by the displacement of several water molecules from the active site on trisaccharide binding. This phenomenon was also observed by Ford *et al.*[14] in the $(NAG)_3NAL$ difference map. Using the coordinates of water molecules associated with lysozyme as deposited in the Brookhaven Data Bank, we find that there is a water position 1.15 Å from C_2 of ring B and a second water position 2.11 Å from C_8 of ring D. Displacement of these two water molecules results in the poor distribution of electron density near these two atoms of MGM. A total of 10 water molecules are probably displaced by MGM upon binding to lysozyme; the distances between nearby atoms of the trisaccharide and these water positions in the native structure vary from 0.8 to 2.1 Å.

A second major feature of the difference map is a concerted shift of the main chain and side chain atoms from Gly 71 to Leu 75, which is relayed from the active site region via a hydrogen bonding network which includes Ser 72 O, Ser 60 O, Asn 59 Oδ1 and Arg 61 N. Concommitant with the binding of trisaccharide, the indole ring of Trp 62 moves towards site B and Trp 63 towards site C into a possible H bonding position with the carbonyl oxygen atom of the 2-acetamido group of NAG. A small shift of Trp 108 away from the substrate binding region is also suggested by this difference map. The magnitude of the main chain movement was obtained by fitting the difference density with the affected residues as a rigid unit on the graphics system. The difference in the coordinates between the native conformation and the shifted position was 1.8 Å. Similar conformational changes in these same regions of the lysozyme molecule have been described[23]. The binding of several acetamido monosaccharides have elicited movements of up to 1.0 Å in the region of amino acids 70–76. The shifts described by Perkins *et al.*[23] are, however, considerably smaller than the 1.8 Å movement indicated for those residues as a result of MGM binding.

The conformation of the active site of lysozyme with MGM bound is illustrated in Fig. 2. The most intimate contact between the substrate and the enzyme occurs in site C with the nitrogen of the 2-acetamido group hydrogen bonded to the carbonyl oxygen of Ala 107. Other stabilising hydrogen bonds and close contacts between MGM and lysozyme are listed in Table 1. This table also contains, for comparative purposes, those close contacts between lysozyme and the tetrasaccharide lactone[14]. The lactyl side group of the sugar in site B is in close contact with the native position of the guanidinium group of Arg 125 of a neighbouring molecule (related by $\frac{1}{2}-y, \frac{1}{2}+x, \frac{3}{4}+z$). However, there is clear indication in the difference map of the disruption of the intramolecular ion-pair between Asp 119 and Arg 125 which is present in the native lysozyme structure but prevented from forming when MGM is bound. Coordinates for the fitted model of MGM have been deposited with the Brookhaven Data Bank.

Catalysis by hydrolytic enzymes is described most simply as the lowering of the activation energy barrier of the rate limiting step by the enzyme providing a surface complementary to the substrate geometry at the transition state. How this lowering of the activation energy barrier is achieved is the subject of much discussion. It is quite likely that there are a number of factors involved, some more important than others. The lysozyme mechanism has run successively through several of these factors[3,4,9,10], and it has been suggested that electrostatic stabilisation is the most important contributor to the rate enhancement[11,12]. Stabilisation of the positively charged carbonium ion intermediate of the NAM residue in site D of lysozyme is made by the carboxylate ion of Asp 52, with little distortion of the glucopyranose ring in site D away from the $^4C_1$ chair conformation. On the other hand a crystallographic study of the tetrasaccharide lactone, NAG_3NAL, complexed to lysozyme[14] shows that the δ-lactone is in a conformation close to a sofa and in this conformation the ring atoms C1, C2, C4, C5, O5 are coplanar. It would appear that the lactone does mimic the previously proposed[4] carbonium ion intermediate[14]. The measured association constant of $3.3 \times 10^6\ M^{-1}$ for the lactone is also supportive of this view[24].

The relationship of the binding of the transition state analogue[14] (NAG_3NAL) to the binding that we observe for the trisaccharide MGM is shown in Fig. 3. Also included in Fig. 3 are the positions of the two catalytic residues Glu 35 and Asp 52. It is evident from this Fig.3*a* that the whole trisaccharide penetrates much less deeply into the active site cleft of lysozyme than does NAG_3NAL. The root mean square deviation of the 43 common atoms between these two molecules in their respective binding modes is 1.23 Å (sites B, C and D). The view in Fig. 3*b*

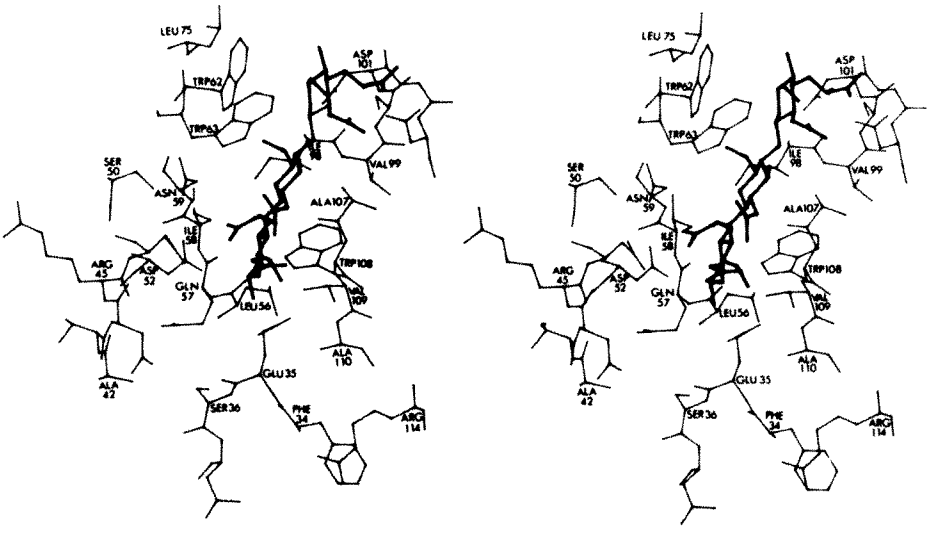


Fig. 2 The active site region of lysozyme with NAM-NAG-NAM bound (heavy lines).

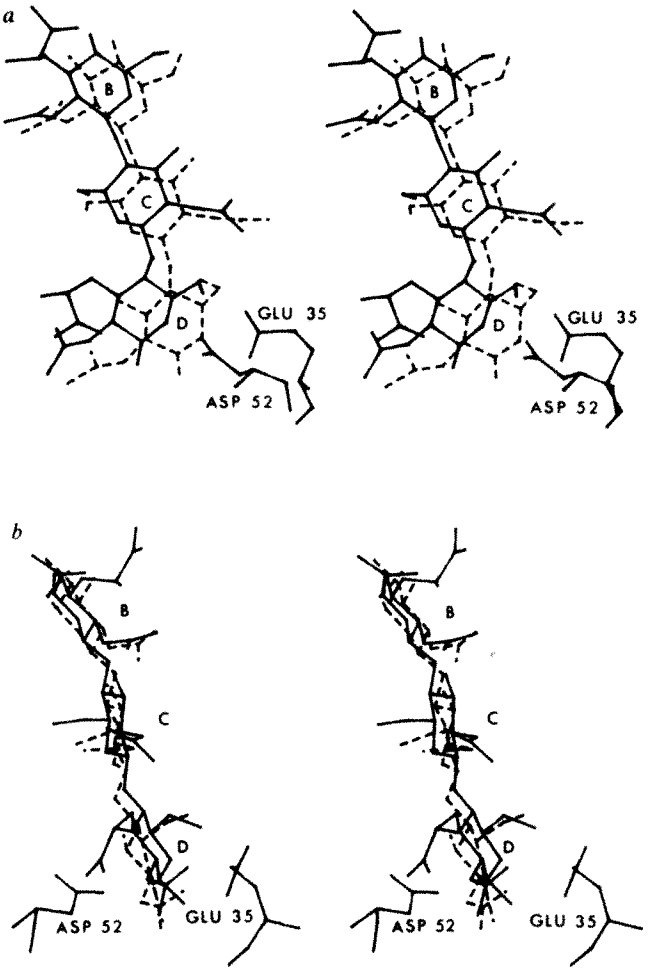


Fig. 3 The difference in the binding modes of MGM (solid lines) and NAG_3NAL (dashed lines) to the active site cleft of lysozyme. Only those residues in sites B, C and D of the tetrasaccharide NAG_3NAL are indicated. The active site residues Glu 35 and Asp 52 have been included to establish the relative orientation of these saccharides to the lysozyme molecule. Comparison of the two orthogonal views *a* and *b* shows that the tetrasaccharide transition state analogue NAG_3NAL penetrates more deeply into the cleft than does MGM. The NAM residue in site D is even further out of the binding site than the lactone ring.

indicates that there is little relative displacement in a direction perpendicular to the opening of the cleft. The NAM residue in site D is even further out of the cleft than the corresponding atoms of the lactone. On this basis we suggest that the binding orientation we observe for MGM may be characteristic of an initial Michaelis complex with the enzyme. Holler *et al.*[25,26] have observed the formation of stable productive complexes of chitohexose with lysozyme and have suggested that these complexes are subsequently transformed into reactive complexes. It seems clear that the productive binding and conversion to a reactive complex can only be achieved with the additional binding energy provided by the interaction of two more hexose units to sites E and F on lysozyme. Formation of this reactive complex is therefore associated with general acid catalysis by Glu 35 and electrostatic stabilisation[12] of the ensuing transition state carbonium ion by Asp 52.

The mode of binding we observe for MGM is consistent with a number of facts. The association constant determined by Patt *et al.*[13] is considerably smaller than that of the lactone. Part of this stems from the fact that in the lactone, site A is also occupied by a NAG residue. The remaining differential must stem from the observed differences in the binding modes as described. In the solution NMR study of the binding of MGM to lysozyme[13], it was concluded that there was no distortion of the saccharide bound in site D. The coupling constant studied, however, corresponds to the glucopyranose ring of the NAM residue in site D having the α-anomeric configuration at C1 (approximately 66% of the substrate molecules are α (ref. 27)). Binding of the β monomer could not be observed because the chemical shift of the β-anomeric proton is near the solvent peak in the spectrum. The α-anomeric configuration requires an axial hydroxyl group. However, there is no electron density which would correspond to an axial hydroxyl group (Fig. 1*b*). Thus, it would appear that the active site of lysozyme in the crystal is capable of preferentially binding the β-anomer in site D. Examination of Fig. 2 shows that if an axial OH were present in the *N*-acetylmuramic acid in site D there would be close contacts with the side chains of Asn 46 and Asp 52 which could be relieved, presumably, by a further rotation of the sugar ring out of its observed binding site.

Table 1 Comparison of close contacts between lysozyme and inhibitors*

Site	Protein atom	Substrate atom	Distance with MGM	Distance with NAG_3NAL†
B	Asn 103 Oδ1	O10	2.7	—
	Asn 103 Nδ2	O10	2.2	—
	Asp 101 Oδ2	O 6	4.7	4.3
C	Ala 107 O	N 2	2.0	3.9
	Trp 108 Cδ2	C 8	3.8	4.0
	Trp 108 Cε2	C 8	3.5	3.2
	Trp 108 Nε1	C 8	2.9	2.6
	Trp 108 Cδ1	C 8	2.7	3.2
	Trp 108 Cγ	C 8	3.4	4.2
	Gln 57 O	C 8	3.7	3.6
	Trp 63 Nε	O 3	4.0	3.4
	Asn 59 N	O 7	4.2	2.8
D	Asp 52 Oδ2	C 5	3.4	2.7
	Asp 52 Oδ2	O 5	3.5	2.0
	Asp 52 Oδ2	C 1	3.6	2.4
	Val 109 Cγ1	C 2	3.5	3.7
	Val 109 Cγ1	O 5	2.9	4.5
	Val 109 Cγ1	C 1	3.4	4.1
	Val 109 Cγ1	O 6	3.1	3.3
	Val 109 N	O 6	3.4	3.4
	Asn 46 Nδ2	N 2	4.1	3.0
	Asn 46 Nδ2	O10	3.0	—
	Glu 35 Oε1	O 6	2.8	3.3

* Distances to native coordinates.
† From Ford *et al.*[14].

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Quasi-hexagonal molecular packing in collagen fibrils

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Collagen molecules in native 66.8 nm (*D*) periodic fibrils are widely believed to be assembled into discrete, rope-like sub-structures, or microfibrils[1–17]. Several types of microfibril have been proposed (2, 4, 5, 7- and 8-stranded), mainly on the basis of information contained in the medium angle X-ray diffraction patterns of native tendon fibres[1,5–7]. These patterns show a series of equatorial and near-equatorial Bragg reflections which indicate that the collagen molecules are arranged on a three-dimensional crystalline lattice. The 4-stranded[11], 5-stranded[4–6] and 8-stranded[7,8] microfibrils are *D*-periodic with approximate diameter 3.8 nm, and these and the 2-stranded[10] model are supposed to be packed on a three-dimensional lattice whose basal unit cell, (approximately) perpendicular to the fibril axis, is tetragonal (or quasi-tetragonal) with side *a*, $a\sqrt{2}$ or $2a$, where *a* is ~3.8 nm. In this paper we describe a re-interpretation of the X-ray data[5,6] which leads to a new model for the crystalline regions of the fibril, based on quasi-hexagonal molecular packing without microfibrillar sub-structures, and hence having the character of a molecular crystal[18].

The crystallographic implications of combining hexagonal molecular packing[1,19–25] with the necessity for intermolecular $n \times D$ staggers[26,27] have been discussed[28,29]. Structures with regular D (and $4D$) or $2D$ (and $3D$) staggers in the three principal directions give rise to several types of crystallographic unit cells[29]; that suggested by McFarlane[28] has Bragg plane separations showing some agreement with the observed X-ray diffraction row line spacings[29]. However, some strong X-ray reflections are not indexed and, for those that are indexed, the discrepancies between observed and calculated spacings are greater than for the tetragonal indexing schemes. We now show that this type of model can be modified to give a good fit with the X-ray diffraction pattern. The X-ray pattern shows the most intense Bragg reflections on row line spacings $1/1.369\ \mathrm{nm}^{-1}$, $1/1.328\ \mathrm{nm}^{-1}$ and $1/1.264\ \mathrm{nm}^{-1}$ and, if it is assumed that these three spacings correspond to the principal Bragg plane separations in a slightly distorted (quasi) hexagonal packing scheme (Fig. 1), the agreement between the positions of the row lines predicted by the resulting unit cell and the observed data is excellent (Table 1). The unit cell is $a = 2.667$ nm, $b = 3.903$ nm, $\alpha = 104.58°$ and 17 of the 19 observed spacings are indexed with a standard deviation between observed and calculated reciprocal (or diffraction) spacings[17] of $0.004\ \mathrm{nm}^{-1}$ (compared with $0.010\ \mathrm{nm}^{-1}$ for the tetragonal indexing[6] of the same spacings). The two other, non-trivial combinations of the three principal quasi-hexagonal spacings give rise to unit cells which do not correspond so well to the observed data. The new unit cell predicts fewer unobserved row lines, in the range of the observed data, than any of the 5.4 nm or larger tetragonal cells. Furthermore, the strong intensity on the three row lines of approximate spacing $1/1.3\ \mathrm{nm}^{-1}$ is a natural consequence of the three principal Bragg plane separations in the quasi-hexagonal lattice.

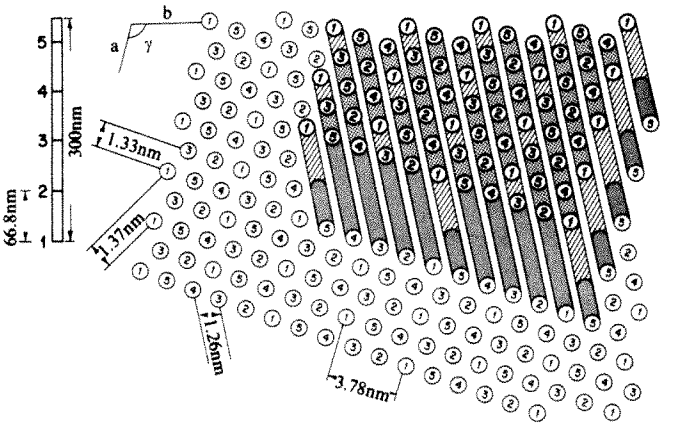


Fig. 1 Possible structure in one region of a collagen fibril, shown in projection down the fibril axis. Numbers refer to molecular segments (see upper left) in one 66.8 nm (D) thick transverse section. Thick shaded lines show molecular tilt and connectivity between upper (bold type) and lower (regular type) surfaces of the section. The molecular polarity and sign of the tilt (~5°) are arbitrary. In this example the lateral shift of the unit cell origin in height D is $\Delta a = a/10$, $\Delta b = -3b/10$. The same unit cell dimensions could also arise by regular combinations of two $1D$ (and $4D$) staggers and one $2D$ (and $3D$) stagger in the three principal directions, as well as by more irregular patterns of intermolecular $n \times D$ staggers.

The fanning of the near-equatorial reflections in the X-ray diffraction pattern indicates that the collagen molecules are inclined at a small angle to the fibril axis, either by tilting of straight molecules or by super coiling[5]. In the present model we propose straight molecular tilting, in such a way that the axes of the molecules lie in the 1.264 nm separated Bragg planes (Fig. 1). The molecules then appear tilted, by about 4°, when viewed perpendicular to the fibril axis along either the 1.369 nm or 1.328 nm separated Bragg planes. The tilting then accounts for the observed intense equatorial $1/1.264\ \mathrm{nm}^{-1}$ reflection and the intense off-equatorial reflections on the $1/1.369\ \mathrm{nm}^{-1}$ and $1/1.328\ \mathrm{nm}^{-1}$ row lines. Since the diffraction pattern arises from a large number of azimuthally disorientated fibrils, the off-equatorial reflections appear symmetrically on either side of the equator.

In general, hexagonal molecular packing of straight tilted molecules with intermolecular $n \times D$ staggers implies a lateral

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shift in the unit cell origin in height D when viewed in projection down the fibril axis. This corresponds to lateral (perpendicular to the fibril axis) shearing of the three-dimensional unit cell and, for a given molecular tilt, the direction and extent of shearing is determined by the unit cell contents. Since lateral shearing of the unit cell in real space (the structure) corresponds to axial shearing (parallel to the meridian) of the reciprocal cell in reciprocal space (the diffraction pattern), reflections on the various row lines will be displaced parallel to the meridian. This effect could account for certain row line reflections indexing as non-integral orders of D, as is observed, for example, on the 1/3.8 nm^{-1} row line[5,6], though more detailed calculations will be necessary to account fully for features such as this. Lateral shearing and molecular tilt are illustrated in Fig. 1.

Table 1 Indexing of observed rat tail tendon row line spacings[6] with unit cell based on quasi-hexagonal molecular packing

1/observed (nm)	1/calculated (nm)	h	k
3.800	3.778	0	1
2.651	2.582	1	0
2.461	2.436	1	−1
1.890	1.918	1	1
	1.889	0	2
1.748	1.748	1	−2
1.369	1.369	1	2
1.328	1.328	2	−1
1.264	1.264	1	−3
	1.259	0	3
1.224	1.218	2	−2
1.141	1.137	2	1
1.031	1.034	1	3
0.964	0.969	1	−4
	0.959	2	2
0.865	0.870	3	−2
	0.861	3	0
0.815	0.823	1	4
	0.812	3	−3
0.72	0.718	3	2
0.68	0.685	2	4
	0.680	1	5
0.63	0.632	2	−6
	0.630	0	6

Equatorial reflections have been observed on the 1/1.748 nm^{-1} and 1/0.964 nm^{-1} row lines. We note that these spacings index as $(1, \bar{2})$ and $(1, \bar{4})$ respectively in the present unit cell (Table 1), and thus correspond to Bragg planes which are almost parallel to the 1.264 nm separated Bragg planes $(1, \bar{3})$. Lattice sampling of the tilted molecular transform on these row lines would therefore be expected to give reflections very close to the equator, and these reflections may appear equatorial as a result of specimen disorientation (1.5°). This model therefore gives explicit account of the equatorial or off-equatorial occurrence of the most intense reflections in the near-equatorial part of the X-ray diffraction pattern.

The preceding discussion has assumed that the row lines are parallel to the meridian of the X-ray pattern. The data from native fibres, however, shows a small angular splitting for several row lines which indicates axial shearing in the structure[5–7,17]. From the identical angular splittings (±3°) of both the meridional and 1/3.8 nm^{-1} row lines[17], this shearing must be centred about the a axis. These data are only consistent with tetragonal lattices if there is no intensity on two of the four predicted 1/3.8 nm^{-1} row lines[17], whereas no such problem arises for the quasi-hexagonal model, in which only two 1/3.8 nm^{-1} row lines are predicted.

Observations in the region of the 1/0.95 nm^{-1} layer line (from the collagen triple helix) allow the relation between the molecular tilt and the axial shear to be specified. For native fibres, the intersection of the inner 1/3.8 nm^{-1} row line with this layer line occurs at a smaller distance from the equator than the intersection of the outer row line. This implies that the row lines are inclined in a direction opposite to that of the molecular tilt, as projected on to the plane of the axial shear. This interpretation is confirmed by the behaviour of these reflections during the so-called orthomorphic transition[5,17], in which the angular splitting of the row lines is reduced. The transition is accompanied by a shift towards the equator of both reflections representing the intersections of the 1/3.8 nm^{-1} row lines with the 1/0.95 nm^{-1} layer line, as predicted.

Density measurements offer a possible method of discriminating between the different molecular packing models[29]. These data are somewhat confused, however, by the relative insensitivity of the high angle equatorial X-ray spacing to the water content, in the region of 1/1.3 nm^{-1} (that is, when the 3.8 nm lattice is observed)[30,31]. Since it is difficult to imagine how water could enter the fibril without altering the equatorial X-ray spacing this observation has been interpreted as representing an increase in the interfibrillar water content[30]. Thus, taking a lower limit for the water content at an equatorial spacing of 1/1.31 nm^{-1} (that is, the centre of the '1.3 nm triplet') of 0.6 g water per g collagen[31], and assuming an average dry collagen density[30,32,33] and intrafibrillar water density of 1.36 g cm^{-3} and 1 g cm^{-3} respectively, the proportion of the intrafibrillar volume occupied by collagen in native rat tail tendon fibrils is approximately 55%. Table 2 shows that the relative exclusion volumes of the various packing models are distributed over a wide range, with the (quasi) hexagonal models showing the best agreement with the observed data. Independent support for hexagonal packing has been obtained using a molecular probe technique[24].

Table 2 Relative exclusion volumes for the different packing models

Basal unit cell			Model	Volume of fibril occupied by collagen
a (nm)	b (nm)	γ(deg)		
3.847	3.847	90	Tetragonal packing of 5-stranded microfibrils[6]	37%
5.440	5.440	90		37%
7.694	7.694	90		37%
7.56	7.55	90	Tetragonal packing of 5-stranded microfibrils[17]	38%
2.7	4.12	109	Hexagonal packing of molecules[27]	57%*
2.667	3.908	104.56	Quasi-hexagonal packing of molecules	54%
2.62	4.02	109	Hexagonal packing of molecules[28]	55%*
5.5	5.5	90	Tetragonal packing of molecules[18] or 2-stranded microfibrils[10]	72%*
3.49	3.84	90	Tetragonal packing of 8-stranded microfibrils[7,9]	82%*

* Assuming the c axis is perpendicular to the a–b plane and equal to $n \times 66.8$ nm.

It should be emphasised that here we account only for the positions of the sharp Bragg reflections in the near-equatorial part of the X-ray diffraction pattern. The breadth of these reflections measured in a direction parallel to the equator indicates that the lateral extent of long-range order is ~100 nm (ref. 5). However, at least 50% of the intensity scattered by the specimen in the near-equatorial region lies between the Bragg reflections in the form of continuous diffuse scatter. The profile of this diffuse scatter[39] falls sharply away from the origin and

reaches a broad maximum at about $1/1.3\ nm^{-1}$. Since fibril diameters in adult rat tail tendon are in the range 8–550 nm (ref. 34), it may be concluded that some fibrils or parts of fibrils are crystalline and other fibrils or parts of fibrils are disordered. Alternatively the disorder may exist within the crystalline lattice itself. As the Bragg reflections show very little increase in breadth (as measured parallel to the equator) with increase in distance from the origin, this and the form of the diffuse scatter are indicative of a mixed crystal[35]—a crystal in which the crystal lattice is well developed but throughout which the unit cell contents vary. Variation in the azimuthal positions of the molecules in the unit cells would cause diffuse scattering. To determine whether this variation is static or dynamic requires measurement of inelastically scattered radiation in the Rayleigh or Brillouin regions[36] or measurements of nuclear magnetic resonance[37] which already have been interpreted as suggesting dynamic azimuthal movement of the molecules in wet tendon.

Therefore, based on a new interpretation of the X-ray diffraction patterns, we propose that, in the collagen fibrils of tendon, the molecules are tilted to the fibril axis and packed on a quasi-hexagonal lattice that is both laterally and axially sheared. Discrete, rope-like, microfibrillar sub-structures within the fibril are not required by the X-ray data, though the unit cell does contain the minimum D-periodic descriptive unit[38]. The data do not preclude specific cross-linking patterns which may define minimum structural units[3,11].

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X-ray diffraction from the side-by-side model of DNA

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An intriguing topological problem posed by the double-helical Watson–Crick[1] model of DNA is that of unwinding the intertwined strands during replication. Several workers have recently proposed novel side-by-side (SBS) structures for DNA[2–6]. In all these models the two strands are joined by complementary Watson–Crick base pairs and the antiparallel polynucleotide strands alternate between short segments of right- and left-handed helix, thus both reducing the amount of intertwining and alleviating the unwinding problem. We show here that there are unacceptable discrepancies between the observed diffraction pattern of B-DNA[7] and that calculated for the original SBS structure[2]. We also describe a simple modification of this model which resolves some of the more serious discrepancies. However, the agreement is still markedly inferior to that obtained for a Watson–Crick model of DNA.

The model of Rodley *et al.*[2] is the only SBS structure for which detailed coordinates have been published. This model can be considered as most similar to the B conformation of DNA and Rodley *et al.* claim that it can account for patterns of the B type. The B conformation has been observed in both crystalline fibres of lithium (LiDNA) and semicrystalline fibres of sodium DNA (NaDNA). After allowance has been made for the effects of molecular packing, the X-ray scattering from a DNA molecule in NaDNA is, within the limits of the data from these fibres, essentially identical to that from one in LiDNA, indicating a very similar if not identical molecular structure. There is, therefore, a compelling requirement that any SBS model be capable of accounting not only for the data from semicrystalline patterns but also the more extensive and precise data from crystalline fibres.

Rodley *et al.* proposed their model as typical of a whole family of SBS structures, the members of which might differ, for example, in the number of nucleotide pairs in the right- and left-handed regions. However, the crystalline B X-ray data are compatible with the orthorhombic space group $P2_12_12_1$. The symmetry elements of this group when combined with the non-space group systematic absences (when $h+k$ is odd and $l=3n$, where n is integral) indicate that each DNA molecule must contain at least one diad axis per five nucleotide pairs perpendicular to the helix axis and orientated along the a or b axis. This extra symmetry element requires that the number of nucleotide pairs in the right- and left-handed segments be equal.

In contrast to the claim by Rodley *et al.*, the value of the reliability index (for the agreement between the SBS model and the observed diffraction data) calculated by Arnott[8] suggests that the SBS model is in substantially worse agreement with observed data than the best model of the Watson–Crick type. It is not clear that a single parameter such as the reliability index is the best way of comparing models of this kind and we compare below the full molecular Fourier transforms of the SBS and Watson–Crick models. This approach allows the regions of the diffraction pattern where the most significant discrepancies occur to be identified and provides an essential basis for any consideration of possible structural refinement of the SBS model.

The model of Rodley *et al.* does not completely solve the unwinding problem as it is still helical. With a minimal distortion of the original coordinates, the structure may be treated as a 10-fold right-handed helix with 10 nucleotide pairs per residue and a pitch of 345 Å. There is 2-fold rotational symmetry within each residue so that the structural asymmetric unit consists of

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five nucleotide pairs. The diffraction from such a structure is confined to layer planes with spacing $1/345$ Å^{-1}. Both the method of calculating the Fourier transform[9] and the correction for the effect of water in the fibre[7] have been described elsewhere.

A detailed comparison of the diffraction calculated for the SBS model proposed by Rodley *et al.* and that observed for the B form is illustrated in Figs 1 and 3. The diffraction pattern in Fig. 1 is in very close agreement with unpublished calculations of D. R. Davies (personal communication). It is immediately clear that this particular SBS model cannot account for the X-ray diffraction from crystalline fibres of LiDNA. This is because the translational repeat along the helix axis for the SBS model is 345 Å, whereas that required by the X-ray data is 34.5 Å (the value is actually 33.7 Å but this difference is not relevant to the argument). The only possibility for removing this major discrepancy would lie in the extremely unlikely event of a model of this type having a Fourier transform on all layer planes for which $l \neq 10p$ (where p is any integer, including zero) which was accidentally zero. Figure 1 shows that such a fortuitous occurrence is not the case for the published model coordinates.

The translational repeat of the SBS model would, however, be reduced to 34.5 Å (and the predicted diffraction thus confined only to layer planes which are actually observed) if the rotation per residue were 0° instead of 36°. A model with no net rotation may be thought to be biologically more attractive as the polynucleotide chains do not then intertwine at all. We have, therefore, applied a uniform distortion to the published SBS model to reduce the net rotation to 0°. This was achieved by reducing the atomic ϕ coordinates within the qth nucleotide of the repeating unit by $\frac{35}{10} \times (q-1)°$. This is a crude way of modifying the coordinates but it is quite satisfactory for examination of the calculated diffraction expected from this sort of model. The modified model can be regarded as a helical structure with one residue per helix pitch. As for the original SBS model, the residue consists of 10 nucleotide pairs with 2-fold symmetry. The transform of this model is plotted in Fig. 2.

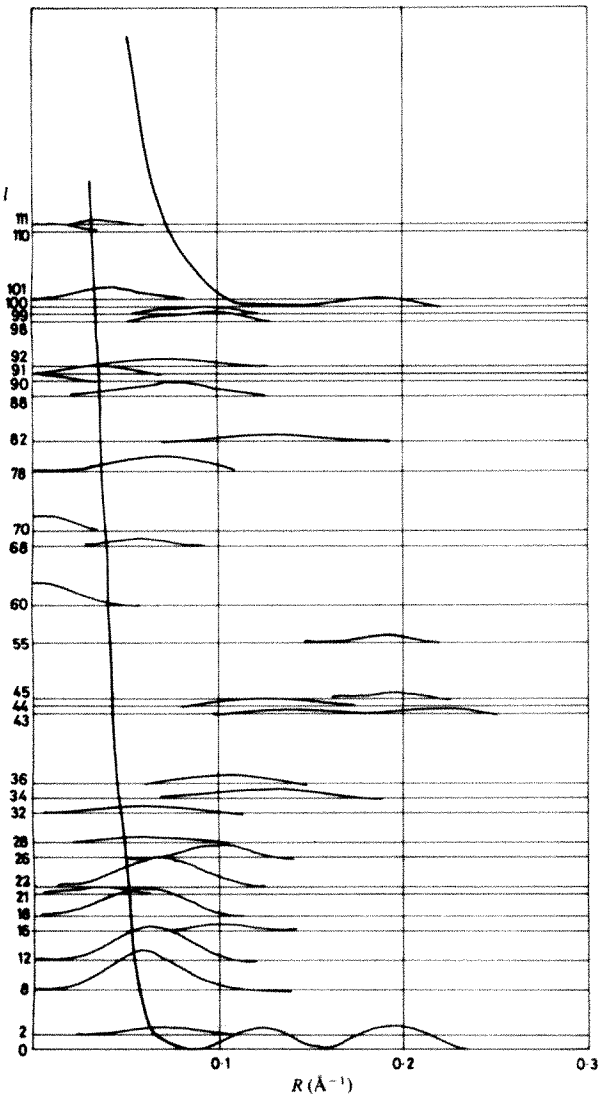


Fig. 1 Cylindrically averaged squared Fourier transform for the side-by-side model for DNA proposed by Rodley *et al.*[2]. The transform on each layer plane l is plotted as a function of the distance R from the centre of the plane.

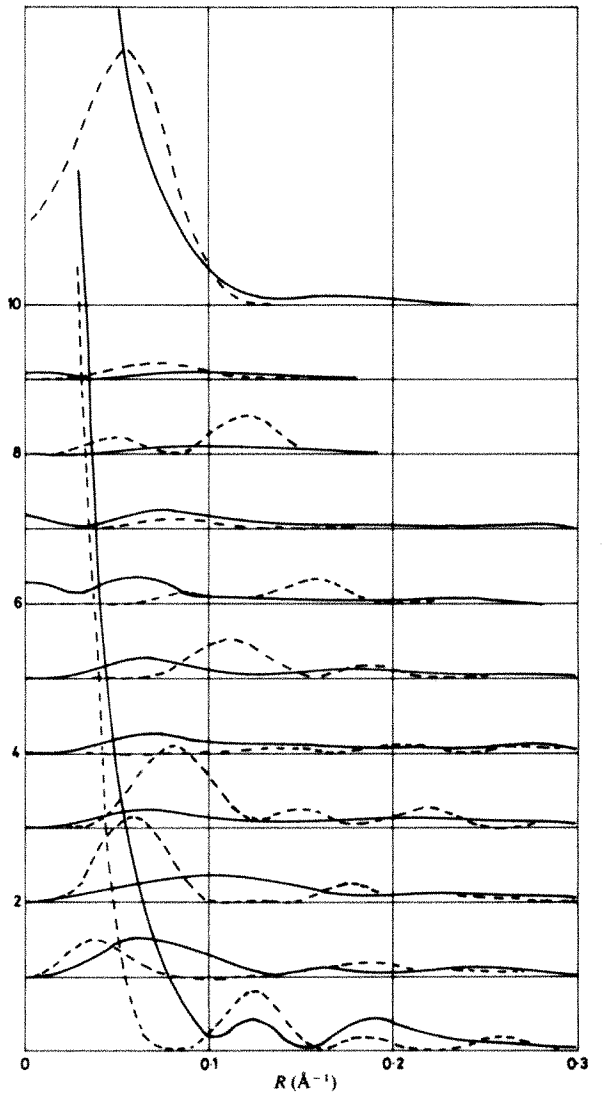


Fig. 2 Comparison of the cylindrically averaged squared Fourier transform of a distorted simplified version of the original side-by-side model (solid curve) with that of the Watson and Crick model for DNA described by Arnott and Hukins[10] (broken curve).

Figure 3 represents the level of agreement which was obtained between observed and calculated diffraction in the initial refinement of the Watson–Crick structure of crystalline LiDNA. The dashed curves in Fig. 2 represent the calculated diffraction for a further refinement of this structure using the linked-atom least-squares technique[10]. While there are significant differences between the diffraction calculated for these two

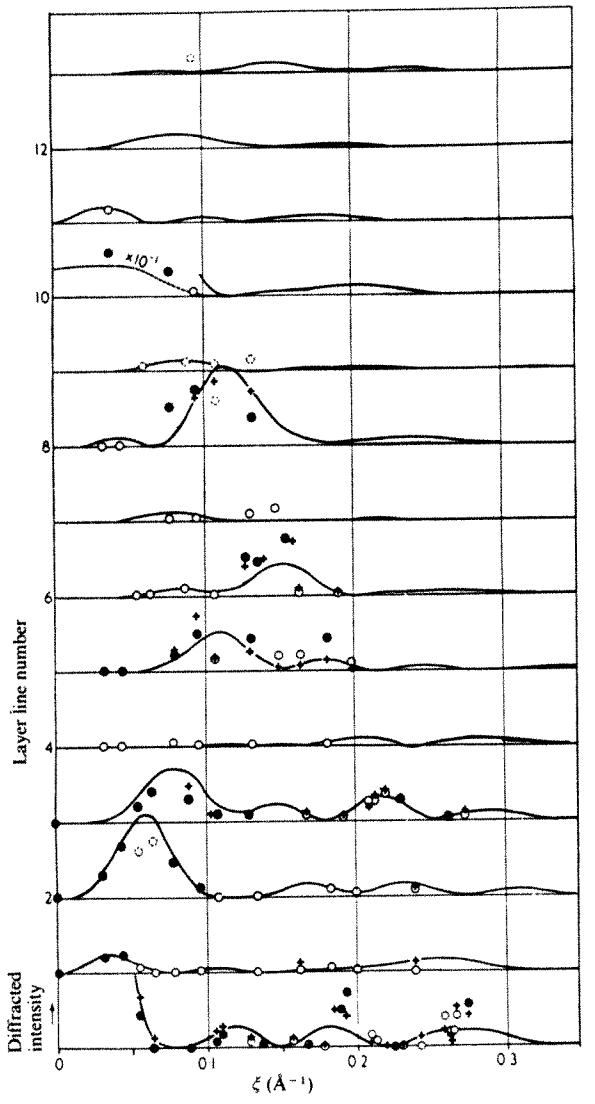


Fig. 3 Comparison of the cylindrically averaged squared Fourier transform of the Watson and Crick model for DNA refined by Langridge *et al.*[7] with the observed intensities. Observed intensities in order of decreasing reliability are indicated by ●, ○ and ◌. ξ is the distance from the centre of the layer line and is equivalent to R in Figs 1 and 2.

Watson and Crick models, the differences are not large and for both models the calculated diffraction is similar to that observed. Even the most superficial examination of Figs 1–3 reveals that the diffraction calculated for the two SBS models differs markedly from that observed.

A particularly serious deficiency of the original SBS model is the occurrence of diffraction on layer planes which are neither observed nor predicted by the Watson–Crick models, that is on $l = 8$, 12, 18, 22, 26, 78 and 88 in Fig. 1. There is no doubt that the quality of the X-ray diffraction patterns from crystalline fibres of LiDNA is sufficient for the original SBS model to be eliminated on the basis of the non-observation of these layer planes. The SBS model also predicts substantial meridional diffraction on layer planes $l = 60$, 70 and 90 (corresponding to $l = 6$, 7 and 9 in the normal B-DNA nomenclature). This is also at variance with the observed diffraction. In addition to predicting diffraction which is not observed, the SBS model fails to predict the strong diffraction which is observed on layer planes $l = 10$, 20, 30, 50, 60 and 80 ($l = 1$, 2, 3, 5, 6 and 8 in the normal nomenclature).

It can be seen from Figs 2 and 3 that while the distorted SBS model only predicts intensity on those layer planes for which diffraction is actually observed, the magnitudes of these predictions are seriously in error. Specifically, substantial meridional diffraction is predicted but not observed on layer planes $l = 6$, 7 and 9. Furthermore, the distorted model is seriously in error in its prediction of the relative intensity of diffraction on the various layer planes. These discrepancies are particularly serious on $l = 2$, 3 and 8 where the overall calculated intensity is much less than is observed and on $l = 4$ for which substantial diffraction is predicted where the observed intensity is essentially zero.

We may conclude that there are major and quite unacceptable discrepancies between the observed diffraction from the B form of DNA and that calculated for the SBS model proposed by Rodley *et al.*[2]. Although some of the more serious discrepancies can be removed by the simple distortion of the original model described here, the degree of agreement between observed and calculated diffraction is still very poor and very much inferior to that reported for the best models of the Watson–Crick type. It should also be emphasised that DNA can change reversibly within a fibre from a conformation that gives the semicrystalline B pattern to one that gives a fully crystalline A pattern[11]. This change is easily explained in terms of the Watson–Crick model by an increase in the number of nucleotide pairs per pitch from 10 to 11. Even if an SBS model could be found to account for the B diffraction data, it would also need to be capable of undergoing a stereochemically plausible transition to account for the A diffraction pattern.

This work was supported by the SRC.

Received 3 August; accepted 30 October 1979.

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Errata

The authorship of the article 'Crust of oceanic affinity in Iceland', *Nature* **281**, 347 (4 October) emerged incorrectly as a result of a misunderstanding between us and our typesetters on the telephone. It should have read:

Scientific Party, Iceland Research Drilling Project (I. L. Gibson, editor).

We regret this error.

In the letter 'Chlorophyll and nitrate fine structure in the southeastern Bering Sea shelf break front' by R. L. Iverson *et al.*, *Nature* **281**, 664–666, the units mentioned in the legends to Figs 3 and 5 should read: (μg atoms NO_3–N l^{-1}).

In the obituary to Dr Scott Mazzur, *Nature* **280**, 708, the new antigenic marker mentioned in the penultimate paragraph is the 'l' antigen, not the 'e' antigen as shown.

Synopses of the British Fauna (New Series) No. 17
edited by D. M. Kermack and R. S. K. Barnes

British Brachiopods

Keys and Notes for the Identification of the Species

C. Howard C. Brunton and Gordon B. Curry
January 1980, vi+64pp., £4.80 $11.50 0.12.357550.8

Today Brachiopods can be regarded as 'living fossils' found inter-tidally and in deeper water, from below antartic ice to tropical reefs. *British Brachiopods* deals with the British represenatives of this group of marine animals. Although less diverse today than in past ages, there are 21 distinct species found in the waters around the British Isles, all of which are described in this synopsis. Often called 'lamp-shells', brachiopods have been confused with bivalve molluscs. The book shows how this confusion can be avoided as the orientation of the shells is quite different in the two groups of animals. This synopsis will be of great interest to zoologists, palaeontologists and naturalists, and will encourage the search for these living remnants of a great fossil record.

Interferon Volume 1

Editor-in-chief: Ion Gresser
Board of editors: K. Cantwell, E. de Maeyer, M. Landy, M. Revel and J. Vilček

December 1979/January 1980, xii+164pp., £9.80/ $23.00 0.12.302250.9

INTERFERON has been introduced to provide a much-needed forum specifically for investigators from all disciplines working on interferon. This publication is neither a journal reporting the latest experiments nor an 'annual review', although it is intended that it will appear once a year. Rather the focus will be primarily on 'position papers' by international experts which will reflect the major issues and dominant themes in interferon research. The style will be personal and informal, and contributors will be encouraged to interpret for a wide audience their ideas, current work, aims and problems, to discuss and clarify controversial points, and to speculate on the prospects of future work. For anyone concerned with interferon this publication is a vital new source of information on the progress made, current issues and the goals still to be attained.

Experimental Archaeology

John Coles

December 1979/January 1980, c320pp.
Hardback: £10.80/ $25.00 0.12.179750.3
Paperback: £4.80/ $11.50 0.12.179752.X

This book picks out the major trends in experimental archaeology but the choice of work described is selective and represents the author's interest in archaeological experiment as an important means of retrieving and explaining evidence about early societies. The first chapter is an historical treatment of experimental archaeology, questioning the evidence and devising new approaches. The following chapters look at ocean voyages, the production of food and the building of houses, the manufacture and use of tools and weapons, achievements in arts and music, the erection of monumental structures for the dead and, finally, modern attempts to experience 'life in the past'.

Aspects of Consciousness

Volume 1: Psychological Issues

edited by Geoffrey Underwood and Robin Stevens
January 1980, xvi+252pp., £12.40/ $29.00

The two editors of this book are both psychologists, one with leanings towards the cognitive approach, the other following the physiological approach. This has resulted in a well-balanced book with diverse opinions represented as how best to describe consciousness. It also helps to indicate the common ground held by cognitive and physiological explanations in a variety of closely associated areas of research. This volume of *Aspects of Consciousness* brings together a variety of discussions on mental processes. They range across a broad spectrum of topics which are of current interest in psychology. Chapters on traditional subjects such as memory and thinking, discussions on relationships between sensory deprivation and consciousness, hypnosis which can be considered an altered state of consciousness and time perception. Theoretically interesting aspects of consciousness are also covered in chapters on the development of consciousness and absent-minded behaviour.

Energy Risk Management

edited by G. T. Goodman and W. D. Rowe

December 1979/January 1980, c400pp., £14.80/ $34.50 0.12.289680.7

The topics discussed represent selected thematic contributions made at the International Review Seminar held in September 1978, by the International Institute for Energy and Human Ecology of the Royal Swedish Academy of Sciences, and the Swedish Energy Research and Development Commission. Risk assessment problems are considered under five separate themes. The first part of the book broadly reviews the risk assessment approach — its advantages and its drawbacks — and highlights the more important problems for detailed treatment in the remaining four parts. The second part examines some of the special problems connected with risk data and their interpretation, while the third part deals with the difficult question of the so-called "real" risk assessment as opposed to perceived or intuitive reaction to risk by the public.

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Behçet's Syndrome

Clinical and Immunological Features

edited by T. Lehner

December 1979/January 1980, xii+324pp., £16.40/ $38.00 0.12.442680.8

Behçet's syndrome is the term used to describe a combination of clinical features that almost always include oral ulceration and are associated with genital ulceration, skin lesions, arthritis, inflammation of the eye, and vascular and neurological manifestations. The aim of this book, which records the proceedings of a conference sponsored by the Royal Society of Medicine in London, is to assist the large number of specialists who may encounter one or more of the manifestations of Behçet's syndrome to make a correct diagnosis. The contributors describe in detail the various clinical manifestations, discuss the diverse therapeutic approaches and deal at length with recent advances in the immunological investigation of Behçet's syndrome.

Cognitive Processes in Spelling

edited by Uta Frith

January/February 1980, c550pp., £17.00/ $39.50 0.12.268660.8

The book opens up a new field of study on cognitive-linguistic skills, their development and their abnormalities. Dealing with English orthography — which is highly complex and therefore an ideal testing ground — it analyses many different aspects of the subject, including both word production and word recognition in spelling. It looks at spelling, as a skill and as a problem, in children, adult illiterates and highly practised spellers as well as in children with special problems such as dysphasia, deafness and dyslexia. Collections of spelling errors from normal and abnormal samples are provided for the reader to analyse, as well as new diagnostic tests. The comprehensive bibliography will be a useful guide to further research.

A Volume in the Mathematics in Science and Engineering Series

Applications of Functional Analysis and Operator Theory

V. Hutson and J. S. Pym

December 1979/January 1980, c400pp., £17.00/ $39.50 0.12.363260.9

This book guides the non-specialist through the beginnings of the subject towards both the principal achievements in linear theory and to certain areas of current concern in nonlinear theory — arguably the area where in practice functional analysis makes its most important contribution. The book has two aims. Firstly, through a careful selection of material, it provides essential abstract techniques for applications. In order to make this material more accessible the work contains more explanation than is normal in standard functional analysis texts. Secondly, the text shows how the abstract theory will work in practice. Gradually the theory is exploited to tackle problems which occupy a central position in applications. The book concentrates on what might roughly be described as "the solution of equations".

Advances in Microbial Physiology Volume 20

edited by A. H. Rose and J. Garreth Morris

January/February 1980, x+368pp., £19.60/ $45.50 0.12.027720.4

This series presents critical review articles on all aspects of the physiological and biochemical activities of organisms. *Advances in Microbial Physiology*, spanning the area between microbiology and biochemistry, is able to deal with subjects of interest in both fields which previously have received little attention from reviewers.

Contents

W. Ford Doolittle: The cyanobacterial genome, its expression and the control of that expression.
M. Young and J. Mandelstam: Early events during bacterial endospore formation.
Hiroshi Nikaido: The outer membrane of gram-negative bacteria.
Stephen A. Morse, Anthony F. Cacciapuoti and Paul G. Lysko: Physiology of *Neisseria gonorrhoeae*
Author index. Subject index.

Fibrous Proteins: Scientific, Industrial and Medical Aspects Vol. 1

edited by D. A. D. Parry and L. K. Creamer

January/February 1980, xviii+508pp., £19.80/ $46.00 0.12.545701.4

This is the first of two volumes based on the "International Conference on Fibrous Proteins: Scientific, Industrial and Medical Aspects", held at Massey University, Palmerston North, New Zealand, from 12-16 February, 1979. Fifteen eminent scientists were invited to present reviews of the current state of progress in their respective specialist fields. These reviews have been collected together in this volume, presenting the most comprehensive résumé of the subject available. The scientific, industrial and medical aspects of each of the following four subject areas are considered: muscle and meat, collagen and leather, keratin and wool and finally, "other fibrous proteins" such as elastin, fibrinogen and silk. The conference coverage ranged from the protection of wool from the degradative effects of bright sunlight, to recent advances in the sequencing and secondary structure of collagen.

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Renewable Energy

Brent Sorensen

December 1979/January 1980, xviii+684pp., £19.80/ $46.00 1.12.545701.4

Bent Sørensen provides a much-needed source of reference for the facts, methods, principles and interrelations in the renewable energy field. The book discusses the origins of the renewable energy resources and gives models for assessing the environmental impact of their use. It estimates how much there is of each, and how efficiently energy can be extracted by different means, by evaluating the principal methods of converting renewable energy. It further discusses the composition and operation of energy systems, including renewable energy converters, storage facilities and an eventual fuel-based back-up system, and gives examples of system simulations. Finally, the adaption of economic theory to allow a fruitful socio-economic discussion of long-range energy planning alternatives is investigated.

Plants and Islands

edited by D. Bramwell

January/February 1980, x+460pp., £24.00/ $55.00 0.12.125460.7

Many scientifically valuable plants and animals are under serious pressure from the activities of man. This book is based on a symposium entitled Plants and Islands, held in Las Palmas de Gran Canaria in April 1977. Its aims are to draw attention to the scientific importance of islands, to review the present state of knowledge of insular floras, and to emphasize the necessity for further research on the taxonomy and biology of island plants, before it is too late. This book brings together, for the first time, the opinions of specialists on the origins, evolution, systematics and conservation of insular floras on a world scale. It will therefore provide a most useful source of reference for biologists interested in evolution, biogeography and conservation.

The Physiology and Pathophysiology of the Skin Volume 6

edited by A. Jarrett

December 1979/January 1980, c320pp., £27.20/ $63.00 0.12.380606.2

Volume 6 of this much-praised series continues the tradition of earlier volumes by providing reviews of literature within the broad field of dermatology. The range of topics already covered is extended by this volume to include, in three sections, the mucus membranes, the action of vitamin A on mucus membranes and the skin, and transepidermal water loss. Dermatologists, anatomists and physiologists, as well as those working in the fields of dentistry, gynaecology and food science, will benefit immensely from this comprehensive review. Medical and biological librarians and research workers in all these subjects will also welcome the recent findings described in this work, some of which are reported for the first time.

Form and Function in Birds Volume 1

edited by A. S. King and J. McLelland

December 1979, xii+470pp., £28.00/ $64.50 0.12.407501.0

This extensively illustrated, three-volume work is intended to provide a definitive account of avian morphology, surveying the principal anatomical features and showing how they work. The survey is not confined to the domestic species but covers birds generally. Evolutionary factors influencing avian morphology have been included where they help to clarify avian form. The overriding objective has been to show why birds are built as they are, rather than to provide an encyclopaedic catalogue of descriptive anatomy. The authors, drawn from Europe and North America, have adopted individual approaches in their chapters, placing varying emphasis on physiology, comparative vertebrate anatomy, and gross and microscopic anatomy; for purposes of continuity, however, the editors have tried to establish a reasonable uniformity of style. The anatomical terminology is based on the *Nomina Anatomica Avium*.

Nomina Anatomica Avium

An Anatomical Annotated Dictionary of Birds

edited by J. J. Baumel

January/February 1980, c600pp., £28.00/ $64.50 0.12.083150.3

The vast amount of anatomical detail and variety of form to be found in avian species has always suggested the need for a codified scheme of names for the various systems and anatomical parts of birds. With this in mind, the International Committee on Avian Anatomical Nomenclature was established in 1971 with a membership that soon grew to include some 80 eminent avian scientists. Their objectives in compiling the *Nomina Anatomica Avium* were to improve communication among avian scientists and to advance our anatomical knowledge of birds. To this end they adopted the universally acceptable language of Latin, chose one name where two or more are in current use, produced new names where none was available, replaced names that were grossly defective or inappropriate and codified the prevailing usage of satisfactory names.

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Tropical Botany

Proceedings of a Symposium held at the University of Aarhus, 10-12 August 1978

edited by Kai Larsen and B. Holm-Nielsen

December 1979/January 1980, xii+452pp., £28.80/ $66.50 0.12.437350.X

This book contains a series of papers on tropical botany covering a rich field of knowledge, from plate tectonics and palynology and their importance for understanding the evolution of floras, to topics such as dispersal, evolution of species and pollination biology. For the first time all these subjects have been drawn together in relation to tropical vegetation, the most endangered plant community on the world's surface today. Contributors include outstanding botanists from major botanical centres such as the Royal Botanic Gardens, Kew, London; the Smithsonian Institution and The New York Botanical Garden; Laboratoire Phanerogamie, Paris; the Rijksherbarium, Leiden; and the Universities of Tokyo and Kyoto in Japan.

Advances in Electronics and Electron Physics No. 52

Seventh Symposium on Photoelectronic Image Devices

edited by B. L. Morgan and D. McMullan

January/February 1980, xvi+532pp., £32.60/ $75.50 0.12.014703.3

This well established publication is now edited to a different brief, resulting in a shorter but more concise and authoritative work. The Seventh Symposium was held at the Imperial College, London in September 1978. These symposia are the most important international conferences in this rapidly developing field. The papers give an account of the state of the art in detection and analysis of images by photoelectronic devices. Recent developments in television camera tubes and image intensifiers are included as well as the very important progress now taking place in solid-state detectors such as charge-coupled diode arrays and charge-injection devices. Professor Wilcock has written an introductory paper giving an overall view of the changes which have taken place in the field of photoelectronics during the last twenty years.

Proceedings of the Serono Symposia Series No. 15

Haemostasis and Thrombosis

edited by G. G. Neri Serneri and C. R. M. Prentice

December 1979/January 1980, xiv+800pp., £35.00/ $80.50 0.12.516150.6

This book is the edited proceedings of the first Florence Conference on Haemostasis and Thrombosis. This was a major meeting where specialists in the field of thrombosis and haemostasis convened to discuss advances in both the clinical and biochemical aspects of the subject. The wide range of topics discussed included blood coagulation — in particular fibrinogen and factor VIII biochemistry, interactions between platelets and vessel walls, fibrinolysis and its inhibition, effects of oral contraceptives, platelet pharmacology, and the current forms of treatment of clinical vascular and thrombotic disorders. This volume will provide a most useful source of reference for research workers in this area while physicians, surgeons and biochemists working in allied areas, will find here a comprehensive review of this important subject.

The Neurobiology of Dopamine

edited by A. S. Horn and B. H. C. Westerink

January/February 1980, xviii+724pp., £36.00/ $83.00 0.12.354150.6

The book ranges widely in its coverage, from the comparatively simple chemistry of the dopamine molecule to the complexities of its role in human mental illness. Between these extremes there are sections on the analysis of dopamine and its metabolites, on its biosynthesis, anatomical distribution and synaptic activity, and on the neurophysiology, behavioural functions and clinical aspects of dopamine containing systems. Each contributor has treated his topic at a level which will be comprehensible to those who are not specialists in that particular discipline. Anyone working on dopamine can now consult this book to learn of the full range of research recently carried out on all aspects of its neurobiology.

Modern Physics in Chemistry Vol. 2

edited by E. Fluck and V. I. Goldanskii

January/February 1980, xiv+638pp., £37.00/ $85.50 0.12.261202.7

Like its predecessor, this second volume aims to emphasize the contributions that the techniques described have made to the solution of chemical, physico-chemical and biological problems, rather than to detail methodology. The techniques offered apply to such fields as X-ray photoelectron spectroscopy, neutron diffraction, secondary ion-mass spectrometry, Mössbauer spectrometry and nuclear magnetic resonance spectroscopy. Some of the chapters deal with methods which have already found broad applications and here their authors draw the reader's attention to potential usage as well as providing references for more established modes of employment. Others cover methods — such as X-ray photoelectron spectroscopy and secondary ion-mass spectrometry — which have not yet found an entrance into chemistry on a broad scale and these chapters should break new ground.

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Applied Fibre Science
Volume 3

edited by F. Happey

December 1979/January 1980, xx+552pp., £37.80/ $87.00 0.12.323703.3

Applied Fibre Science is the first work to provide a comprehensive survey of the methods used to study polymers and fibres, the appropriate areas of application of these methods and the present state of knowledge. The full range of polymers and fibres is covered, including the man-made and natural fibres used in the textiles, paper and other industries, the polymers produced in the plastics industry, as well as biopolymer research and its development in the field of molecular biology. Experts in the particular fields, selected on an international basis, have contributed to the work, giving it the broadest authority. Volume 3 is mainly concerned with the work that has been done on specific types of polymers and fibres.

Organophosphorus Reagents in Organic Synthesis

edited by J. I. G. Cadogan

January/February 1980, c580pp., £38.80/ $89.50 0.12.154350.1

In addition to the considerable practical importance of organophosphorus compounds for their own sake, for example as pesticides, antioxidants, or plasticizers, they have enormous value as general reagents in organic synthesis. It is to provide a summary of these applications that this monograph has been written. Considering the voluminous amount of literature on organophosphorus chemistry, it is all the more surprising that no such work of reference has yet been published. All researchers engaged in organic synthesis, industrial laboratories and university chemical libraries are bound to find this an invaluable work of reference.

Announcing a New Journal from Academic Press

European Heart Journal

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Publication: Bi-monthly

Subscription: Volume 1, 1980
£23.00 (UK) $58.00 (overseas)

The journal is primarily intended for the publication of original work in cardiovascular medicine and surgery but important components of the journal will be reviews and notes in relation to recent developments in the field, for example, new drugs and techniques. It will publish reports from the European Society of Cardiology and its Working Parties and also from other supranational bodies such as the World Health Organization. Although published in English, it will be an international journal with its base in Europe. There will be periodic reviews of the non-English language literature from Europe and correspondence and book reviews will be regular features. *European Heart Journal* will prove an invaluable source of information for all those concerned with cardiology and it will take its place alongside other journals on this subject as an authoritative and scholarly work.

Contents of Volume 1 will include:

R. Michels, M. Haalebos, P. P. Kint, F. Hagemeiger, K. Balakumaran, M. Brand, P. W. Serruys, E. Bos and P. G. Hugenholtz: Intra-aortic balloon pumping in severe angina and infarction

G. R. Sutherland, R. H. Anderson, R. Heslope and S. Hunter: The value of sub-xiphoid approach in ultrasonic examination of the atrioventricular junction

Also: Report of the Working Party on Thallium and Blood Pool Imaging Collaborative Trial in the Prevention of Coronary Heart Disease, by the WHO European Collaborative Group.

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Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy

by **G.C. Levy**, *Florida State University*
and **R.L. Lichter**, *Hunter College, City University of New York*

This describes the characteristics and methodology, treating in detail ^{15}N chemical shifts, spin-spin (scalar) couplings, and relaxation parameters. In order to serve the needs of a wide variety of readers, the book is organized so that the qualitative discussions are followed by more extensive and quantitative treatments. It includes major developments appearing before February 1979, with several scientists providing previously unpublished material.

October 1979 — 232 pages
0471 02954 8 — $23.50/£10.75

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by E. **Charney**, *National Institutes of Health, USA*

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and **G.A. O'Connor**, *New Mexico State University*

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by **A.F.M. Barton**, *Murdoch University, Western Australia*

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reviews

Longing for simplicity

P. D. Moore

Plant Strategies and Vegetation Processes. By J.P. Grime. Pp.222. (Wiley: Chichester, UK, and New York, 1979.) £11.50.

IF there is one straw for which all biologists grasp, it is a clear conceptual framework upon which to hang the varied observations which form the raw material of biological research. No biological mind has equalled that of Darwin when it comes to the discovery of common denominators within disparate facts, but many have used a similar approach with varying degrees of success. In a science in which laws are statistical and exceptions are often commoner than the rule, it takes a brave man to make firm statements concerning the way in which living organisms are structured or behave in relation to their environment. That such statements are readily latched on to by the academic masses is evidenced by the current popularity of such concepts as *r* and *K* selection, or island biogeographic theory. We all long for simplicity in nature and embrace it firmly when it is shown to us.

J.P. Grime of the University of Sheffield is one of those people who has actively sought simple statements which can be made about nature and in particular about the various strategies which have evolved within the Plant Kingdom and which confer certain advantages upon those species possessing them. He conceives of three basic strategists: competitors, stress tolerators, and ruderals. He defines competition as the tendency of neighbouring plants to make use of the same resources; the competitor strategist is thus a species which, as a consequence of robust stature, fast growth rate and optimal timing of canopy expansion, is well fitted to obtain at least its fair share of those resources. Stress is defined as the external constraint upon the growth of part or all of the plant; the stress tolerator is thus a species which can continue its growth in conditions which might be expected to curtail the growth of others. Ruderal species are characteristic of disturbed habitats, where disturbance is defined as an interruption to plant biomass accretion. On the basis of this view of plant species, Grime proposes that three selective forces, *C* selection, *S* selection and *R* selection, are operative which had led to the evolution of the observed strategies. In attempting to reconcile this system with the currently prevailing *r*–*K* selective spectrum, Grime proposes that *R* selection is equivalent to *r* selection, *S* selection to *K* selection and that *C* selection occupies the intermediate part of the continum.

Having three, rather than two, defined endpoints in a classificatory scheme means that a simple one-dimensional ordination of species along a line is no longer appropriate and that, instead, one must think in triangular terms (an analogous model to the triangle of texture in pedology). In this way we can admit such bastard strategists as "stress-tolerant ruderals" and even "*C*–*S*–*R* strategists" which combine some features of all strategy types.

This system Grime regards as appropriate for any consideration of plants in their established phase. Upon it one must superimpose a further set of subdivisions based upon regenerative strategies, such as vegetative spread, soil seed banks, and so on. Having thus defined and explained his terms, the author then proceeds in the second part of the book to put them to the test, applying them to a consideration of various features and processes found in vegetation, such as dominance, coexistence and succession.

The real test of the value of this novel concept of vegetation is, of course, whether it assists in the explanation and understanding of observed phenomena. In this respect there are many pleasing features about Grime's model. One of its most successful applications, to my mind, is in the explanation of the high species density found in calcareous grassland, where low phosphorus and nitrogen availability can be regarded as a stress which limits the development of *C* strategists and permits the proliferation of *S* strategists and various non-competitive intermediate types.

Perhaps the least satisfactory aspect of the system is the loose definition of stress. The problem here is that what represents a stress for one species may be beneficial for another (take water-logging, for example). As most ecologists think of species as possessing bell shaped performance curves along environmental gradients, stress really has to be defined separately for each taxon, in terms of its optimum and performance limits. It cannot be defined in general terms.

Grime claims that his model is consistent with Odum's suggestion that the greatest diversity occurs in the middle range of a physical gradient, but this is not so. Odum considered that where conditions were least extreme, diversity would be highest. Grime claims that in such locations the *C* strategists would have a hey-day and plant species density would be low. Higher diversities would thus be achieved as one moves out from the centre towards physical extremes (there must be a better expression than "stress-gradient" for these axes). In this respect Grime's view seems to accord with reality more closely than Odum's.

This book is stimulating, refreshingly novel, controversial, perhaps a little oversimplified in places, but has one most valuable feature: it forces the reader's mind along new paths. He may not always completely agree with the author's views and conclusions, but he is sure to benefit from examining them. This freshness of approach, coupled with a concise and lucid style make this book a valuable tool for teachers of plant ecology. □

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Large scale magnetic fields

Cosmical Magnetic Fields. By E.N. Parker. Pp. 841. (Clarendon/Oxford University Press: Oxford, 1979.) £45.

ALTHOUGH the same basic physical principles apply to cosmical magnetised plasmas as to those produced in the laboratory, very significant differences arise in their application. Trapping of plasma in the laboratory is carried out with magnetic fields anchored to rigid conductors, and the aim is to produce equilibrium configurations. Astrophysical fields are trapped by gas, however, and gravitational forces give rise to magnetic buoyancy: dynamical activity seems to be the norm, rather than stable equilibrium, because of this absence of anchoring. These magnetic fields play important roles in determing conditions throughout the Universe, as they control the production and flow of the cosmic radiation, affect the accumulation of interstellar gas into clouds, and determine the radiation intensity of bremsstrahlung sources. It is clear that theoretical work would have been aimed entirely at understanding equilibrium configurations of magnetised plasmas in the cosmos if spatially unresolved sources had been the only ones

which could be studied. Observations of the Earth's magnetosphere and of the Sun demonstrate clearly that such studies are, however, too restricted: examination of these objects shows changes in magnetic structure in progress on a whole range of timescales which includes the 22 year magnetic cycle of the Sun and flares lasting minutes. These observations have provided an invaluable stimulus to the theoretician, though it is unfortunately the case that the changes observed are usually so complicated that it is difficult to decide which particular processes cause them.

Although many papers present detailed treatments, books on this rapidly developing subject have tended to illustrate the application of magnetohydroynamics by treating the basic concepts only. Thus, they have tended to provide brief idealised models of the solar dynamo, solar fluxes, sunspots, and the Earth's magnetosphere. Such treatments have been welcome because they provide insight into the basic problems, but it is necessary for workers to understand magnetic configurations more fully if they are to explain detailed changes in the radiation intensity from pulsar magnetospheres, accretion disks, supernova remnants, and so on. This book by Professor Parker is therefore invaluable because it illustrates clearly how largescale magnetic fields develop.

As mentioned earlier, observations of magnetic configurations on the Sun have stimulated theoretical work. Parker has been in the forefront of this work on interpretation for many years and his papers provide considerable insight about what is happening at various features. This book now provides a more systematic discussion with a series of chapters explaining the various configurations and processes. It must be stressed, however, that the author points out the relevance to non-solar studies, where applicable, and there are chapters devoted to magnetic fields of planets, and the field of the Galaxy.

The book begins with a chapter on the role of magnetic fields in the astronomical universe. This is followed by chapters on the nature of magnetic fields, the basic equations, magnetic stress and energy, and so on. One striking feature observed in high resolution photographs of the Sun is the non-uniformity of the atmospheric plasma, which generally shows a filamentary character. This reflects the presence of flux tubes as opposed to an 'equilibrium', large scale, continuous distribution treated in most theoretical work. Parker examines the formation of these tubes, their stability, and twisting of the structures. Magnetic buoyancy of the tubes and the activity which arises at the solar surface (and even within galactic structure) due to the absence of anchor points are also discussed.

Considerable attention has been given over the years to magnetic configurations where rapid relaxation of stress produces solar flares. This topic is discussed in chapter 15, but Parker also considers magnetic reconnection in any general topology where non-equilibrium fields have formed. The diffusion of flux through fluids of high electric-conductivity was once regarded as too slow for reconnection to be significant, but this has had to be reconsidered following laboratory studies of instabilities.

There are also chapters (18 and 19) dealing with formation of magnetic flux, which is a continuing process, as the primordial field has not remained trapped withing many of the regions which are accessible to observation.

Parker states in the preface that he intended individual sections to be intelligible without too much reference to earlier chapters. To this end, he begins most chapters with a concise and well-referenced discussion which places the particular problem in context. This is followed by a detailed analysis, and the chapter is terminated with a descriptive summary outlining the application of the work. Anyone wishing to follow the calculations will certainly require considerable knowledge of the techniques normally used in the field, but the assumptions made, and the conclusions reached, are set out qualitively in a clear, readily assimilated form. It is hardly the case, as the blurb states, that the non-scientist will find the material readable.

There has been a general reluctance to use SI units in astronomy, but the use of practical units in electromagnetism makes the SI system extemely attractive. In fact, the younger generation of scientists (in British Universities and schools, at least) are not familiar with gaussian units. The reviewer suspects that the adoption of SI by an author of Professor Parker's standing could have accelerated its adoption considerably. He is disappointed that the opportunity has been missed (while recognising that others will disagree), but regards the book as a valuable and stimulating contribution. **W.M. Glencross**

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Social behaviour in insects

Social Insects. Vol.I. Edited by Henry R. Hermann. Pp. 437. (Academic: New York and London, 1979.) $36; £23.40.

GIVEN the role that the social insects (termites, ants, social bees and wasps) have played in the generation of sociobiological theory, a multivolume treatise which summarises our state of knowledge is indeed a welcome addition to the literature. This is the first of three multi-authored volumes. Volume 3 will be devoted to particular groups of insects, with chapters on honey bees, army ants, and so on. Volume 2 and this volume are more heterogeneous in content, with 2 covering communication, general reproductive behaviour, defensive mechanisms, pre-social insects, social spiders, and the systematics of social Hymenoptera and Isoptera.

The present volume consists of eight chapters, held together it seems only by the fact they they all deal with social insects. The authors represent not only a wide array of data, but an incredible diversity of opinion on certain key areas, an example being the "colony as superorganism" concept. I will return to this later.

Chapter 1 (by H.R. Hermann) serves to introduce the series. Hermann provides a brief review of animal behaviour studies and of the various social insects. It is suggested that studies of the genesis of insect social behaviour may be lumped into three categories . . . termed "genetic-behavioral", "serial-behavioral" and "anatomical". The "genetic-behavioral" approach refers to the kin-selection theory of W.D. Hamilton. "Serial-behavioral" and "anatomical" include phylogeneic sequences for the generation of highly social from less social forms. Here are discussed the excellent works of H.E. Evans and C.D. Michener. Although I found it hard to follow all the arguments, the section should be a good introduction to this literature. The conclusion which emerges? I quote from page 16: "Based on the various hypotheses summarized above, phylogeneticists are in obvious disagreement about phyletic associations and the sequence of events that led to a eusocial state in hymenopterous groups". I quote this to emphasise also the word hymenopterous. Isoptera receive barely a mention in this chapter. The chapter concludes with a review of various social traits, such as social dominance and territoriality.

Hamilton's kin-selection theory has been the major stimulation to recent work on the social insects, and this volume includes not just one, but two chapters devoted to genetical approaches to social behaviour. C.K. Starr is more biological, while R.H. Crozier goes more into the formal population genetics. Both chapters pretty much ignore the termites, in favour of the Hymenoptera, with their exotic male haploid (haplodiploid) method of sex determination. There is much overlap in the two chapters but this is a benefit as kin-selection theory is not so well defined as the popular literature would suggest. Both chapters provide good reviews of possible pathways for the evolution of helping or altruistic behaviour. Both provide discussions of haplodiploidy and its possible implications for eusocial behaviour. Crozier explains that haplodiploid population genetics is essentially the population genetics of sex-linked genes and discusses the implications of this for a variety of genetical problems.

These two chapters also treat R.D.

Alexander's "parental manipulation" theory for the origin of eusocial behaviour, but they both make the mechanism seem less likely or more difficult to achieve than is really necessary (see Charnov, *J. theor. Biol.* **75**, 451, 1978, for discussion). Finally, both chapters discuss the recent work of Robert Trivers and Hope Hare on sex ratio evolution within eusocial societies. Crozier's discussion is more detailed, with development of theory and plots of original data. Starr's discussion of the major prediction of the Trivers–Hare theory is incorrect. At the bottom of page 58, he lays out the conditions for the worker's equilibrium investment ratio to be 3:1. The statement should be that the workers lay none of the male eggs, rather than all of them, as he claims. These discussions of the Trivers–Hare theory should be supplemented with readings from more recent literature, omitted from this volume. I suggest particularly Alexander and Sherman (*Science*, **196**, 494; 1977) and Evens (*Bioscience*, **27**, 613; 1977). I found both these chapters quite thought provoking and it is nice to have all this 'genetical stuff' in one place. It should greatly facilitate the intellectual battles which make sociobiology so much fun: I challenge any first year graduate student to resolve the apparent theoretical conflict of Scudo and Ghiselin versus Levitt (see Table 5, page 266 in Crozier).

A short chapter by Carpenter and Hermann discusses the fossil evidence on the social insects. C. Baroni Urbani has contributed a very interesting chapter on applications of the concept of territoriality (a defended area in addition to the nest) to social insects. There seems to be good positive evidence for colony level territoriality in termites and ants. In ants much of the active defense is over food sources, so the territory becomes a defense of certain situations rather than a defense of a certain space or area. Territoriality seems to be rare in bees and wasps, with the exception perhaps of males and their mate search behaviour. Recent work by Johnson and Hubbel (*Ecology*, **55**, 120; 1974), however, has shown defense of food sources by stingless bees (not cited by the author).

G.C. Wheeler and J. Wheeler discuss their several (about five) decades of research into larvae of social Hymenoptera. The chapter contains detailed morphological descriptions and some astute suggestions as to the adaptive significance of various structures. They conclude with discussion on the role of larvae in the colony life. I was also charmed by G.C. Wheeler's following disclaimer "At this point I had better make my customary and necessary disclaimer: I am not related to William Morton Wheeler (the great ant biologist) — that is, not genetically. We were, however, closely related academically. G.C. Wheeler is close to defining a "cultural coefficient of relatedness" roughly defined as the fraction of ideas shared in common by two people. This seems to me to be a useful extension of kin-selection theory.

D.H. Kistner has provided a fascinating discussion of social insect symbionts — the other insects and mites which are found associated in various ways with insect colonies. His discussion of the means by which these guests deceive their hosts, the extent to which some are integrated into the colony life (and others are not, but still manage to keep from being killed) and the evidence on the phylogenic evolution of associations deserves careful study by all students of social behaviour and evolution.

M.V. Brian's chapter on "Caste Differentiation and Division of Labor" is a most imposing document. One hundred pages long with about 400 references, it documents what is known about the proximate control mechanisms for caste structure in all groups of social insects. This chapter fascinated me, both for what it left out as well as what it contained. Let me explain with reference to the concept of "colony as superorganism", a concept which appears throughout this volume. The question is: To what extent may we usefully consider a colony to be a single organism, with the queen as gonads and workers and larvae as somatic tissue geared up to aid the queen's reproduction? Hermann and Starr call it an outmoded and discarded concept. Crozier recognises that the analogy clearly breaks down at times (witness the queen-worker conflict notions of Trivers and Hare) but he is still willing to study theoretical models which explicitly assume it. He cites cogent criticism of the concept by Mary Jane West-Eberhard; criticism directed at E.O. Wilson, whose recent book, *Caste and Ecology in the Social Insects* (Princeton University Press: Princeton and Guildford, UK) with George Oster (for review, see *Nature*, **280**, 519; 1979) certainly does treat the colony as the unit of natural selection. If these authors seem to celebrate the demise of this concept, the chapters by Wheeler and Wheeler, and Brian clearly disagree. This brings me back to Brian's chapter. An excellent discussion of the proximate control mechanisms for caste structure, the chapter never mentions the fact that the reproductive interests of workers and queen must often diverge. Where their reproductive interests do not coincide, we might expect the mechanisms controlling worker behaviour to reflect this conflict and its resolution. Brian presents several instances of caste determination which could be usefully viewed in this way — from the perspective of conflict between workers and queen.

Clearly the authors of this volume (as well as E.O. Wilson and M.J. West-Eberhard) are in conflict over the superorganism concept. If it seems unresolved at present, so be it. Contrary to much popular opinion, the selective forces involved in generation and maintenance of social behaviour of insects are not yet clear. This volume represents very well the present search for underlying principles.

Eric L. Charnov

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Electron image processing

Image Analysis, Enhancement and Interpretation. By D.L. Misell. Pp.305 (North-Holland: Amsterdam, New York and Oxford, 1978.) Paperback $60; Dfl. 135. *Computer Techniques for Image Processing in Electron Microscopy.* By W.O. Saxton. Pp. 289. (Academic: New York and London, 1978.) £15.15.

THESE two books are the first single-author volumes to be devoted wholly to electron image processing, the practical importance of which has become gradually more apparent during the present decade. Despite the similarity in their titles, however, the books are very different in approach and intent. Saxton's is an advanced text, intended for readers already working in the field of digital image processing or for beginners about to immerse themselves in it. Misell's, on the other hand, is addressed to practising microscopists who wish to understand enough about image processing to know whether it could be useful to them. They may be biologists or materials scientists and much of each chapter is therefore written as qualitatively and unmathematically as possible; furthermore, what mathematical reasoning *is* given is cocooned in verbal explanation and definition. Saxton's book requires the mathematical sophistication associated with a degree in physics. One might thus say that Saxton's text is written for students eager to master what he has to offer, whereas Misell's is for an audience reluctantly obliged to acquire some familiarity with this young subject.

Turning to the contents, these too are by no means the same. Misell devotes considerable space to optical methods of processing images, and the book is therefore extensively concerned with periodic specimens. His book opens with a general account of the relationship between a specimen and its electron image, followed by a chapter on optical and computer image analysis, with particular attention to helical structures. A chapter follows on image processing; superposition and filtering techniques being explained in detail. Two chapters are then devoted to contrast enhancement. The first deals with instrumental techniques: defocusing, energy-filtered images, the use of STEM, dark-field and "optical shadowing",

among others. The second describes some simple computer procedures such as subtraction of pairs of images (one highly defocused), and histogram modification. In a final chapter, the author discusses the interpretation of images of various kinds and their reliability.

Misell has gone to great lengths to present all this material simply, with the result that most of the qualitative description will not be found difficult to follow even by readers unfamiliar with the jargon of the subject. The usefulness of the more mathematical parts of the text is much more questionable, however: on p41, for example, it is assumed that the reader will need to be told that "The i which precedes η in Eq. (3.12) is used mathematically to denote a phase shift in the exponential" and that $i = \sqrt{-1}$ (though this is not the first appearance of i). A few pages later, however, he is expected to be able to cope with two-dimensional Fourier transforms in vector notation, convolutions, delta functions and the like. The section on image analysis contains much helpful guidance on indexing diffraction patterns but not really enough to learn the subject: too little for the newcomer, too elementary for the instructed. This criticism is true of much of Misell's book: as a qualitative account of electron microscopy, biased towards periodic biological specimens, it is very clear and contains an immense amount of information; but as soon as an attempt is made to become more exact, it is unsuitable for those with little mathematical background and too elementary for those who may wish to implement the various techniques. Nonetheless, it was certainly well worth making the effort to write a text at this level and much of it will be found extremely helpful.

Saxton's book covers very different ground. An opening chapter is again devoted to generalities about electron image formation, and a short chapter sets out the properties of the discrete Fourier transform. The next five chapters, the heart of the book, are concerned with the question of determining the wave function of which the recorded image indicates only the modulus, the phase being lost. This problem is still being actively explored, though less energetically than during the first few years after the publication of the Gerchberg–Saxton algorithm, the first solution proposed, in 1972. The final three chapters are concerned with the hardware and programming of image processing, and include a description of the high-level language devised by Saxton to simplify the task of image processing on a large IBM computer. However, in view of the numerous subsequent developments and the introduction of a much-improved minicomputer version of the language (SEMPER) by Horner and similar languages in other image processing centres, this material has already drifted somewhat out-of-date. It is surprising that Misell does not deal with this topic at all, for potential users of digital techniques with no time or desire to learn much computing would surely be delighted to learn that simple languages are being devised to enable them to perform advanced processing almost effortlessly.

Saxton's book thus deals very fully with two branches of digital electron image processing: phase determination and picture handling. It is essential reading for anyone studying electron image interpretation, the phase problem in particular, and indeed, the analogous problem that arises in many other fields, optical coherence theory for example. It has the merit that not only is the theoretical support set out fully but numerical methods and computational trials are also described at length. Complicated though the subject is, Saxton has succeeded in writing a clear and highly readable acount and it is unfortunate that much of the intricate work from the Groningen group led by Professor H.A. Ferwerda appeared too late to be fully integrated into the text.

Both books are well-produced, and Misell's is particularly well illustrated, many of the images having been taken specially for this volume. One minor criticism of the latter is the use of "rad" as a radiation unit as well as "radian" — as SI is adopted everywhere else, why is radiation dose not given in grays? The severest criticism that can be levelled at Misell's volume is no fault of the author, however, for the only glaring inaccuracy occurs in the preface. There, Dr A.M. Glauert, the series editor, writes "each book . . . will also be available in an *inexpensive* edition for individual use . . ." (my italics); to describe a 300 page paperback in a successful series costing $60 as inexpensive, while a cloth-bound advanced text of much more limited appeal is priced at (only) £15.15, the series editor must somewhere have lost touch with reality.

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Quantitative study of phytoplankton

Phytoplankton Manual. Edited by A. Sournia. Pp.337. (UNESCO: Paris, 1978.)

THIS book is the latest in a series of monographs on oceanographic technology initiated by the Scientific Committee on Oceanic Research of UNESCO. Although entitled *Phytoplankton Manual* the book is almost entirely devoted to marine phytoplankton, and only four pages are specifically devoted to freshwater phytoplankton.

A group of phytoplankton workers were called together, drew up the general arrangement and content of the volume and then handed over to the editor. In his introductory chapter the editor records the problems involved in drawing together such a work and he also indicates how difficult it is to get contributors to comply with deadlines. Also in the introduction the editor expresses a hope "that the present manual will provide a description and evaluation of all current methods". This is, of course, a fine objective but an impossible one as almost every worker has some refinement or variation which is considered to be an improvement for the conditions found in a particular area. This book does, however, cover another aim of the editor in that "an attempt has been made here to cover the quantitative study of phytoplankton at all stages of research, starting even before the act of collecting a sample (i.e., planning the study) and ending somewhat after the act of enumerating the organisms (i.e. interpreting the results)".

Consisting of a large number of short sections written by people with experience in working with phytoplankton, the methods are described in considerable detail and often compared with related methods, the book is a veritable mine of information. On the other hand because it does contain detailed descriptions of so many methods a young worker just starting in this field might be overwhelmed and find it impossible to decide which is the most suitable method to adopt. It should, however, prevent many of the initial errors which tend to detract from the value of the early results of inexperienced workers.

This, no doubt, will prove to be a valuable manual for all workers in marine phytoplankton, but I imagine a comparable one for the freshwater phytoplankton will also be required. If this book leads to a more uniform approach to the sampling, counting, statistical treatment and calculation of biomass of marine phytoplankton samples, it will have served a useful purpose.

The volume is clearly printed with few errors; it is strongly bound, although a more water-resistant cover may have been an advantage for a laboratory manual. There is an adequate list of references, a list of manufacturers of apparatus and a rather sparse index, but as the editor points out, with a chapter on "How to Use the Manual" the index does not need to be comprehensive. The price for a book of this size and containing so much practical information is extremely reasonable.

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Thermodynamics and statistical mechanics

Thermodynamics and Statistical Mechanics By P.T. Landsberg. (Oxford University Press: Oxford, 1979.) £9.75.

IN this book, Professor Landsberg brings together thermodynamics and statistical mechanics to provide a coherent account of these interrelated branches of physical theory. The two subjects are treated sequentially in the text — a wise decision, as attempts to develop the two side by side usually result in the essential structures of both becoming obscured. The value of classical thermodynamics lies in what it can do without invoking microscopic models and it is best learnt by confining discussion initially to the macroscopic level. The terms of reference of Professor Landsberg's book are those of the physicist. In addition to including those topics common to most standard texts, there are chapters on fluctuations and transport properties, sections on negative temperatures and critical exponents, and introductions to irreversible thermodynamics and the theory of chemical reactions. The text also deals with subjects of current interest such as conversion and conservation of energy, black holes, and the early stages in the formation of the Universe.

Thermodynamics and statistical mechanics are both well established subjects so that any new text can only find justification if it has special merit in excellence of exposition or in its choice of emphasis. This book has some attractive features: sections containing core material are distinguished so that the reader may restrict his study to the central development if he wishes; worked solutions are given to the problems; and there are references to papers and comments on a selection of other books so that topics of interest may easily be pursued. The text is at its best (and it is then very good) in some of the sections dealing with particular applications; examples are the expanding universe, negative temperatures, and energy conversion. The general exposition, however, is unsatisfactory. Professor Landsberg states that he is fascinated by the relationship between physics and mathematics; this fascination, however, has unfortunate consequences in the text. The language used in the basic development of the subjects is highly formal, and mathematical structure frequently precedes physical explanation of what the structure is being erected for. As a result, one sometimes has the impression that physical results are eventually produced rather like rabbits from a conjurer's hat. It was diverting to discover that the phase rule is the same as the Euler formula relating the number of vertices, edges and faces of a convex polyhedron; but knowledge of the fact gave me no new insight into the physical world. Is it necessary or even helpful to the typical student to be faced with density matrices, Hessian determinants, Pfaffians and the signs of symbolic logic? It is, in fact, difficult to imagine the kind of student who, coming with little previous knowledge of thermodynamics and statistical mechanics, could find this a good book through which to learn them.

There are other grounds for criticism. It is surprising to find no mention of thermodynamic temperature, no formal definition of the kelvin, no mention by name of the principle of equal *a priori* probability. It is confusing to find both chemists' wavenumber $(1/\lambda)$ and physicists' wavenumber $(2\pi/\lambda)$ used in different parts of the text. It is disconcerting to find important results such as the Maxwell Relations or general equilibrium conditions derived in problems at the ends of chapters. It is irritating to find the Carnot cycle being used in the text before it is defined, again in a problem.

The book is also disappointing as regards its production. Oxford University Press has chosen, presumably in order to keep down costs, to use photographic reproduction of typescript. This can give a text of entirely acceptable appearance providing that the typescript is meticulously prepared. Here, however, alterations are frequently all too visible; in some cases corrections have been made and symbols inserted in ink by hand; and, in at least one place, there is a gap in the text where words have been deleted.

There is some very good material in Professor Landsberg's *Thermodynamics and Statistical Physics*; but the style of his exposition will make it inaccessible to most students. It is a book to be dipped into by those who already know the subject, not a book to learn it from.

C.J. Adkins

C.J. Adkins is Lecturer in Physics at the Cavendish Laboratory, University of Cambridge, UK.

Obesity models

Animal Models of Obesity. Edited by M.F.W. Festing. Pp. 258. (Macmillan: London, 1979.) £17.50.

SYMPOSIA proceedings are all too often fabulously expensive and hopelessly delayed in publication, so that their only logical home is the library of a well heeled insititute of scientific history. This volume — subtitled *MRC Laboratory Animals Centre Symposium No 1* — is the exception to that rule. Though not cheap, it is, by today's standards reasonable in price, and, to judge from its plastic dustcover, the publishers see its home as much in the laboratory as the library. And there it will be found, for it is an authoratative text which covers a major experimental field in nutrition and is likely to be the only repository for a great deal of useful technical information, like Derek Miller's recipes for energy-dense diets, or David Lovell's outstandingly lucid guide on husbandary techniques for colonies of genetically obese rodents. But it is more than a technical guide, it is a unique review of a fast growing field, and one in which biochemists, geneticists, immunologists and nutritionists all meet, to exchange information and to generate ideas.

The book succeeds in giving the impression of interaction, both because of Dr Festing's careful editorial guidance, reflected in, for example, the frequent cross references between chapters, and because the symposium on which it was based was a fairly parochial, virtually all-British affair, in which all the contributors have closely followed each other's work. This parochiality is a trait which I find stengthens, rather than weakens, the feeling of a debate, and it is made possible by the fact that Britain is a leader in obesity research.

The symposium was orignally entitled *Genetic Models of Obesity in Laboratory Animals* but the change in title here is more than justified by the extent to which a number of authors, particularly Miller, Stock and Rothwell, James *et al.* and Garrow, have turned away from the simple gentic models such as the *ob/ob* or *db/db* mouse and the Zucker rat, and studied 'dietary-induced obesity': that in which no simple genetic component can be discerned. It may be, as many authors claim, that these are more appropriate models for the study of obesity in man, which is, after all, the justification for all the work.

At the same time, however, the simple genetic models have a scientific attraction which increases with every new defect they are shown to possess. This volume brings out the multitude of differences that the single Mendelian recessive *ob/ob* gene gives rise to, and in reading it, it is difficult to resist playing the game that so obviously fascinates many of the authors: to fit this metabolic mélange into the single enzyme defect that genetical orthodoxy requires. The man who solves that puzzle will perform a scientific feat that is in no way diminished by the fact that it will not provide a cure for obesity. It is a prize which is there for the grabbing, and anyone who wants to pursue it could do no better than to start with this eminently readable text.

John Rivers

John Rivers is a Lecturer in Nutrition at the London School of Hygiene and Tropical Medicine, London, UK.

Chemistry of metal complexes

Metal Complexes in Organic Chemistry. By R.P. Houghton. Pp.308. (Cambridge University Press: Cambridge, 1979.) Hardback £21; paperback £7.50.

I ENJOYED reading this book; it is written in a concise manner and is a mine of useful information. Its complex chemistry is a little thin in places, but perhaps the dose is correctly adjusted to the organic chemists whom the book addresses. The author has done a remarkably fine job in bringing together and systematising the vast variety of interesting organic reactions made possible or more specific, catalysed, inhibited or altered in direction by the action of metal containing compounds.

The book is aimed at "advanced undergraduates, graduates at the start of a research career in organic chemistry and practising organic chemists who wish to learn more about the chemistry of metal complexes". It is an excellent book for that purpose, and must represent an enormous amount of work on the part of its author. Its main deficiency is a lack of reference to recent primary literature, obviously a matter of policy, but it does give reference to some 125 well chosen reviews.

In 308 pages it is quite impossible to catalogue all of the organic reactions mediated by metal complexes. Nevertheless the author has made a very comprehensive survey of reaction types -except olefin polymerisation, but Ziegler polymerisation of olefins does receive brief mention (page 244). Each type of organic reaction is profusely illustrated by examples, well set out in display formulae. This means that a mere inorganic chemist such as your reviewer was never at a loss to understand the nature of the organic materials mentioned in the text, even in the very few cases where the name did not convey to him the essential chemical structure intended — for example, "methylmagnesium carbonate" on page 104 is not the probably hypothetical MeMgO.CO.OMgMe, but methyl methoxymagnesium carbonate, MeOMgO.CO.OMe.

The first and second chapters are concerned with general principles of complex chemistry in their application to the explanation of organic reactions mediated by metal complexes. They are very useful chapters with emphasis on electronic effects relating mainly to σ- and π-donation by ligands, π-donation by the metal atom, relative metal-ligand affinities, steric effects, and coordination saturation. They indicate how these affect the egress of organic reagents to the metal, whether as strongly or weakly bound ligands, and how the reagents are polarised or otherwise activated to chemical attack. The rationalisation of the complex chemistry which is desired for the purpose of this book is good. It is only deficient in taking no account of ligand field theory, especially to explain the stabilisation of the higher valent states of such metals as iron, cobalt and nickel, for example, by thio and non-π-donor ligands (page 47) and in the explanation of differences between the light and the heavier transition metals. Some short outline of ligand field effects, with a reference to appropriate reviews by the late Sir Ronald Nyholm or L.E. Orgel would have been appropriate here.

The types of reactions covered in the text are much too numerous to mention in this review. They range from the venerable Fredel-Crafts alkylations catalysed by aluminium chloride to the recently developed homogeneous hydrogenation, carbonylation and decarbonylation catalyses based on the heavier Group VIII metal complexes with tertiary phosphines, carbon monoxide (and so on) as ligands. The use of metal complexes in organic chemical synthesis ranges from the simple separation and purification of organic products as ligands, through mediation in organic reactions by shifting equilibria in favour of the desired product, through ester hydrolyses, bond cleavages especially of the CH bond, subsititutions, isomerisations especially of alkenes, hydrogenations, additions and eliminations of various sorts. They are all there, with a strong emphasis on explanations in terms of established physical-organic chemical mechanisms. These are clearly set out and copiously illustrated.

Although I sense that the author lacks some feel for inorganic chemistry, for example, as in his assertion that aluminium chloride exists as Al_2Cl_6 molecules in the *solid* state (page 127) and his formulation of $[ReCl_3(NMe)(PEt_2Ph)_2]$ with a non-bonding electron pair on the nitrogen atom (page 96), the book is a text which I strongly recommend. It is well researched, organised and printed. It summarises beautifully the types of interesting organic reactions which occur on metal complexes and has a very useful 10-page index. At present-day prices it is good value at £21. I congratulate the author and his publisher on a unique and well produced book in an area of chemistry which has been surprisingly slow in catching the imagination of organic chemists.

Joseph Chatt

Joseph Chatt is Director of the ARC Unit of Nitrogen Fixation and Professor of Chemistry at the University of Sussex, Brighton, UK.

Neurophysiological offerings

Studies in Neurophysiology. Presented to A.K. McIntryre. Edited by R. Porter. Pp.440. (Cambridge University Press: Cambridge and New York, 1979.) £32.50.

THIS book, as it proclaims on its cover, is a collection of offerings from his former collaborators and students to a much-loved and respected Australian neurophysiologist, Professor A.K. McIntyre. Professor McIntyre was successively head of the Department of Neurophysiology at Otago and the Department of Physiology at Monash, which quickly grew into a hugely successful venture. The success of these two departments can quickly be appreciated by a glance down the star-spangled list of contributors to this book, many of whose names would be recognised (with enthusiasm, one hopes) by any undergraduate student of neurophysiology.

As an offering to Professor McIntyre, it must have given great pleasure; but how does it rate as a book about neurophysiology? The main difficulty in compiling such a book for publication as opposed to presentation is obviously to keep a coherent thread running through it, and to achieve consistency of style among the many contributions. Many of the individual contributions are, as one would expect, excellent. There are, for example, clear and elegant reviews of such topics as the vesicle hypothesis (Katz), the mechanism of excitation of carotid body chemoreceptors (Eyzaguirre & Fidone) and the functional significance of changes in extracellular potassium concentration in the central nervous system (Somjen). Other authors have chosen to give much more specialised and technical accounts of particular research projects (for example, Laporte on muscle spindle innervation, and Mubbard on the effects of angiotensin on brain neurones).

Towards the end another abrupt gear change takes place with a long and heady philosophical article by Trevarthen called 'Self-organising systems in psychobiology' (Typical headings: Is a mind biology possible?; Anatomies of imagery and intention; Normal duplicity of brains) which is followed by an essay on Claude Bernard and the *milieu intérieur.*

Though good in places, without a doubt, this collection amounts overall to an indigestible motley which might have been better kept as a private retirement present than expensively dressed up for publication. Unless you are in a sentimental mood, the outlay would be hard to justify.

H.P. Rang

H.P. Rang is Professor of Pharmacology at University College, London, UK.

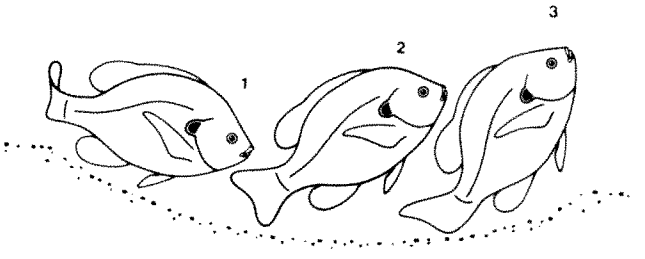
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Biology

M. H. A. Keenleyside

Diversity and Adaptation in Fish Behaviour

1979. 67 figures, 15 tables. XIII, 208 pages
(Zoophysiology, Volume 11)
Cloth DM 69,–; approx. US $ 38.00
ISBN 3-540-09587-X



The book investigates the behaviour of fish and the influence it has on their adaptation to a wide range of marine and freshwater habitats around the world. Topics under discussion are locomotion, feeding, anti-predator behaviour, the organization of fishes within groups, and reproduction. Separate chapters are included on spawning site selection and preparation, breeding and parental care. The book demonstrates the great variability within each of these categories, relating it to the ecological setting of the fish. The first comprehensive work of its kind, this book will be appreciated by ethologists, physiological ecologists, and fisheries biologists.

L. J. Fritschen, L. W. Gay

Environmental Instrumentation

1979. Approx. 76 figures. XVI, 216 pages
(Springer Advanced Texts in Life Sciences)
Cloth DM 42,–; approx. US $ 23.10
ISBN 3-540-90411-5

Up to now, students and specialists in forestry, ecology, agronomy and biological sciences have had to acquire their knowledge of the instrumentation needed for environmental measurements from numerous, scattered sources. *Environmental Instrumentation* presents this information in a unified, systematic treatment.
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Environmental Instrumentation is intended for all those interested in the interaction of biological organisms and meteorological factors. It can be used either as a text for senior level or graduate students or as a reference for environmentalists.



Springer-Verlag
Berlin Heidelberg NewYork

D. M. Gates

Biophysical Ecology

1979. Approx. 163 figures. Approx. 640 pages
(Springer Advanced Texts in Life Sciences)
Cloth DM 79,50; approx. US $ 43.80
ISBN 3-540-90414-X
Distribution rights for India: Narosa Publishing House, New Delhi, India

Biophysical Ecology presents an analytical treatment of the processes by which a plant or animal interacts with its environment. These processes include energy exchange, gas exchange, and chemical kinetics. A plant or animal interacts with its environment by processes which originate outside the organism, flow through the surface of the organism, and ultimately elicit responses through metabolic processes within the organism.
Energy exchange between an organism and the environment involves radiation, convection and evaporation or latent heat. Radiative exchange includes short-wave radiation such as solar, skylight, reflected a sunlight, and long-wave radiation from thermal emission by the ground surface, vegetation, clouds, and the atmosphere. A detailed description is given of the geographical, seasonal, and diurnal dependencies of short and long wave length radiation. The spectral properties of organisms and the environment are described. Analytical models of whole leaf photosynthesis and respiration are presented here for the first time.
Biophysical Ecology will be indispensable to advanced students in all life sciences. It is designed to provide an understanding of the ecology of organisms based on fundamental scientific principles.

Membrane Biochemistry

A Laboratory Manual on Transport and Bioenergetics

Editors: E. Carafoli, G. Semenza
With contributions by numerous experts
1979. 45 figures, 4 tables. X, 175 pages
DM 27,–; approx. US $ 14.90
ISBN 3-540-09844-5

The editors of this book, themselves well-known experts in the field, have assembled a broad spectrum of experiments in membrane transport and membrane bioenergetics. Basic techniques, as well as new and more sophisticated procedures are described in detail, with special attention paid to those which can be applied to a wide range of projects. The methods presented enable even readers with little knowledge of membrane biology to carry out correctly the most important experiments in the field.
The experiments reported have all been successfully tested in the Advanced Courses 29, 41, 45 and 52 of the Federation of European Biochemical Societies for Membrane Biochemistry, Transport and Bioenergetics, organized by the editors at the Swiss Institute of Technology, Zurich, between 1975 and 1978.

Geology

D. A. Ross

Opportunities and Uses of the Ocean

1979. Approx. 145 figures. Approx. 320 pages
Cloth DM 39,50; approx. US $ 21.80
ISBN 3-540-90448-4

Contents: Opportunity of the Ocean. – How the Ocean Works. – The Legal Aspects of the Ocean. – Marine Shipping. – Resources of the Ocean. – Pollution of the Ocean. – Military Uses of the Ocean. – Coastal Zone Divisions, etc. – Innovative Uses of the Ocean. – References.

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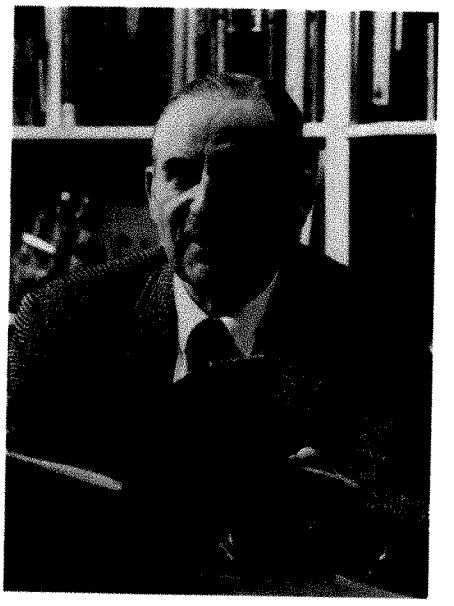
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obituary

Samuel A. Goudsmit, 1902-1978

On 4 December 1978 the world of physics lost one of its most unusual and interesting members when Professor Samuel A. Goudsmit died of a heart attack on the campus of the University of Reno, Nevada. His career was as varied as the times in which he lived; and the changes in science and scientists are accurately mirrored in his actions and his life.

Samuel A. Goudsmit was born in The Hague, The Netherlands, on 11 July 1902; he lived and grew up in a middle class family of merchants. At an early age he showed extraordinary mental agility. He was exceptional in solving puzzles of all kinds. When a young man in high school Goudsmit happened to get a hold of a cheap spectroscope (it presumably belonged to his older sister) and after examining the brilliant spectral colours, Goudsmit was hooked, and spectroscopy and the physics going with it became not only his interest, his hobby and his profession, but a lifelong passion.



Because of his interest first in spectroscopy and later in physics as a whole he started his studies at the University of Amsterdam. After receiving an intermediate degree he went on to obtain his PhD at the University of Leiden. Although Goudsmit's performance was brilliant (with some notable exceptions when he had no interest in the subject) he was considered somewhat of a failure by his family. Physics at that time was a calling, not a profession; with the certainty that there would be no jobs after completing the degree, it was reasonable that Goudsmit's family had a difficult time understanding his devotion to physics. In spite of his unquestioned early successes he was considered as a bit of a misfit.

At the university Goudsmit did well — in physics he did exceptionally well. He was only 19 years old when he pointed out in a paper, (*Naturwiss* **9**, 995, 1921), that the Sommerfeld formula for x-ray doublets was equally valid for alkali doublets. The knowledge, but perhaps even more, the scientific maturity necessary for that observation is highly unusual for a beginning student.

In Leiden, Goudsmit came under the spellbinding influence of Ehrenfest. Ehrenfest was impressed by Goudsmit's talent and interest. The combination of Ehrenfest's insistence on precision and clarity and Goudsmit's natural flair for physics led to Goudsmit's surprisingly rapid development as a scientist. It is therefore understandable that when another one of Ehrenfest's students, George Uhlenbeck, returned to Holland (from a sojourn in Italy) Goudsmit was given the task of bringing Uhlenbeck's knowledge of contemporary physics up to date.

It was in discussions between these two young men, still students, in 1925 that they introduced the notion of "spin". It may well be that by now the familiarity with this concept has blurred the unusual character of their proposal. It took a great deal of knowledge and even more courage to systematically explore and analyze the consequences of this idea. Although Ehrenfest continually encouraged them; the novelty and boldness of this step should certainly be kept in mind.

In 1926 both Uhlenbeck and Goudsmit were offered instructorships at the University of Michigan. There Goudsmit continued his work on the analysis of interpretation on atomic spectra. The field was summarized in an important text on atomic physics written together with Linus Pauling, *The Structure of Line Spectra* (McGraw-Hill, 1930).

Although Goudsmit had been extremely successful in the early phase of his scientific life it would be wrong to say that he was deliriously happy. His unhappiness and moodiness became more pronounced as time went on. There were many reasons for that: one was that (in part, due to his own researches) the field of physics in which he was most expert had reached a certain degree of completion; as such it was no longer in the center of interest. Although Goudsmit later worked in a number of different areas in physics, he never again felt as attuned and in control of a field, as he did in atomic spectroscopy. Although enormously talented Goudsmit often had the feeling that he did not possess enough intellectual technology to function effectively in physics. He had never been terribly interested in mathematics. (He used to say very frequently that he could only understand 2 by 2 matrices and nothing more. This is probably not true, but if it were he certainly made the absolute maximal use of the mathematics he controlled.) Goudsmit often expressed concern about his lack of mathematical ability. He felt that in order to advance in experimental physics a rather detailed understanding of accelerating machinery was essential — that knowledge he did not have either. This made it difficult to choose an effective new research area. There can be little doubt that the dramatic ending of Ehrenfest's life and the calamitous European political situation of the 1930's had a profound effect on Goudsmit. This affected everybody and in particular a sensitive person like Goudsmit. His scientific productivity obviously suffered and this in turn added to his general dissatisfaction with himself and with the world.

It cannot come as a surprise that when the Second World War broke out Goudsmit felt honour bound to take as active a part as possible. He first joined the MIT radiation laboratory and did some work on radar. Later he became the Chief Scientific Officer of the Alsos Project. This was a remarkable scientific intelligence mission; its purpose was to find the extent and status of German atomic weapons research. The results of his mission are written up in a book entitled *Alsos* (Sigma Books, London 1947) which unfortunately is out of print. It describes the mood and the atmosphere of wartime research in Germany. It is also intensely personal and written with great sensitivity — even pathos as when Goudsmit learned that his parents had been deported to an extermination camp. Only those who have suffered the trauma of tragedy and guilt which accompanies the total distruction of a family will fully appreciate the depression that is produced and only they will understand the extraordinarily painful struggle needed to keep going and in fact start anew.

After a short sojourn at Northwestern

University, 1946-1948, Goudsmit joined the Brookhaven National Laboratory in 1948. His interest and devotion to physics was as intense as ever. From 1952 to 1960 he was Chairman of the Brookhaven Physics Department; but equally important was his influence as Managing Editor of the *Physical Review*. He initiated the first letters journal, *Physical Review Letters*, still the most prestigous letter journal in physics.

Goudsmit received many honours, most of which were in connection with the discovery of spin. As expected he shared many of the honours with the codiscoverer of spin, George Uhlenbeck. They received the Research Corporation Science Award (1954), the Max Planck Medal of the German Physical Society (1965), the Karl T. Compton Award for Distinguished Statesmanship in Science, American Institute of Physics (1974) and the National Medal of Science (1977). There was also a commemorative meeting about spin and its discovery at the American Physical Society Annual Meeting in January 1976. Goudsmit and Uhlenbeck got their PhD degrees on the same day, 6 July, 1927. To express the appreciation and admiration of the Dutch scientists a special symposium was held to honour the 50th anniversary of their PhD degrees on 6 July 1977. It was a two day symposium with two highlights: the public highlight occurred when Goudsmit and Uhlenbeck received from the Dutch Government a very high decoration in recognition of their great accomplishments. This was not only a signal honour but a diplomatic feat as well, for such decorations can only be granted to Dutch citizens and Goudsmit and Uhlenbeck although born in Holland, were both naturalized American citizens.

But probably the more impressive and certainly the more touching celebration came when I walked with Goudsmit to the room where 50 years before he had taken his PhD examination. In that old room at the University of Leiden it is a tradition that after passing an examination the students write their names on a wall giving the date and type of examination. Goudsmit had bought a "magic marker" for the occasion; together we walked into the room where he had taken the examination (over the mild objection of the doorman). On the wall were hundreds of signatures. After some searching we found Goudsmit's signature for the right exam at the right time. Right above it Goudsmit wrote his signature with the black magic marker and added "Golden Jubilee of the PhD, July 6, 1977". After that we very quietly walked back. It was very clear to Goudsmit this was a significant and important event.

With the passing of Samuel Goudsmit the world of physics, indeed the whole world has lost a person of great sensitivity, understanding and talent. He was a man of fanatical honesty who detested circumlocution and vagueness. He was the exact opposite of pompous. His standards in physics and in life were extraordinarily high. He made corresponding demands on those around him — on his colleagues, on physics, and most certainly upon himself. That does not make for a terribly easy life, it does not make for a uniformly happy life, but it does mean that one can say with honesty and conviction — it mattered to the world that Samuel Goudsmit lived.

Max Dresden

R.N. Pryor

ROBERT NELSON PRYOR died of a heart attack in London on 14 July 1979, a few days short of his 58th birthday. He was at the peak of his powers and authority as a leading figure in the mining world.

Born in India while his father, a prominent mining engineer, was working in the Kolar Gold Fields, he was educated at Oundle and entered the Royal School of Mines, Imperial College, in 1939. After a year in which he played rugby for the RSM and rowed in the college first VIII, he joined the Royal Engineers and served in India and then with the Chindits in Burma. He finished the war with the rank of Captain, married the daughter of a New Zealand mining engineer, and returned to the College to obtain a first class honours degree in mining engineering in 1948.

His early career had a curious six-yearly cycle. Six years in the army and, when on graduation he went to Rio Tinto Mines in Spain, he spent six years in junior management before being made Chief Mining Engineer — a position which he occupied for six years. He then returned to the London office of Rio Tinto Zinc from where, for six years, he examined new mining projects including potash, lead-zinc, copper, nickel and pyrite in various parts of the world. In 1966 he was invited by the Spanish directors to become General Projects Manager of Rio Tinto Patiño, and in this post he supervised the launching of Cerro Colorado, the largest gold and copper mine in West Europe, and started construction of a new copper smelter and refinery at Huelva. His long and important contributions to Spanish mining did not go unrecognised, for in 1977 he became the first foreigner to receive honorary membership of the Asociación de Ingenieros de Minas de España.

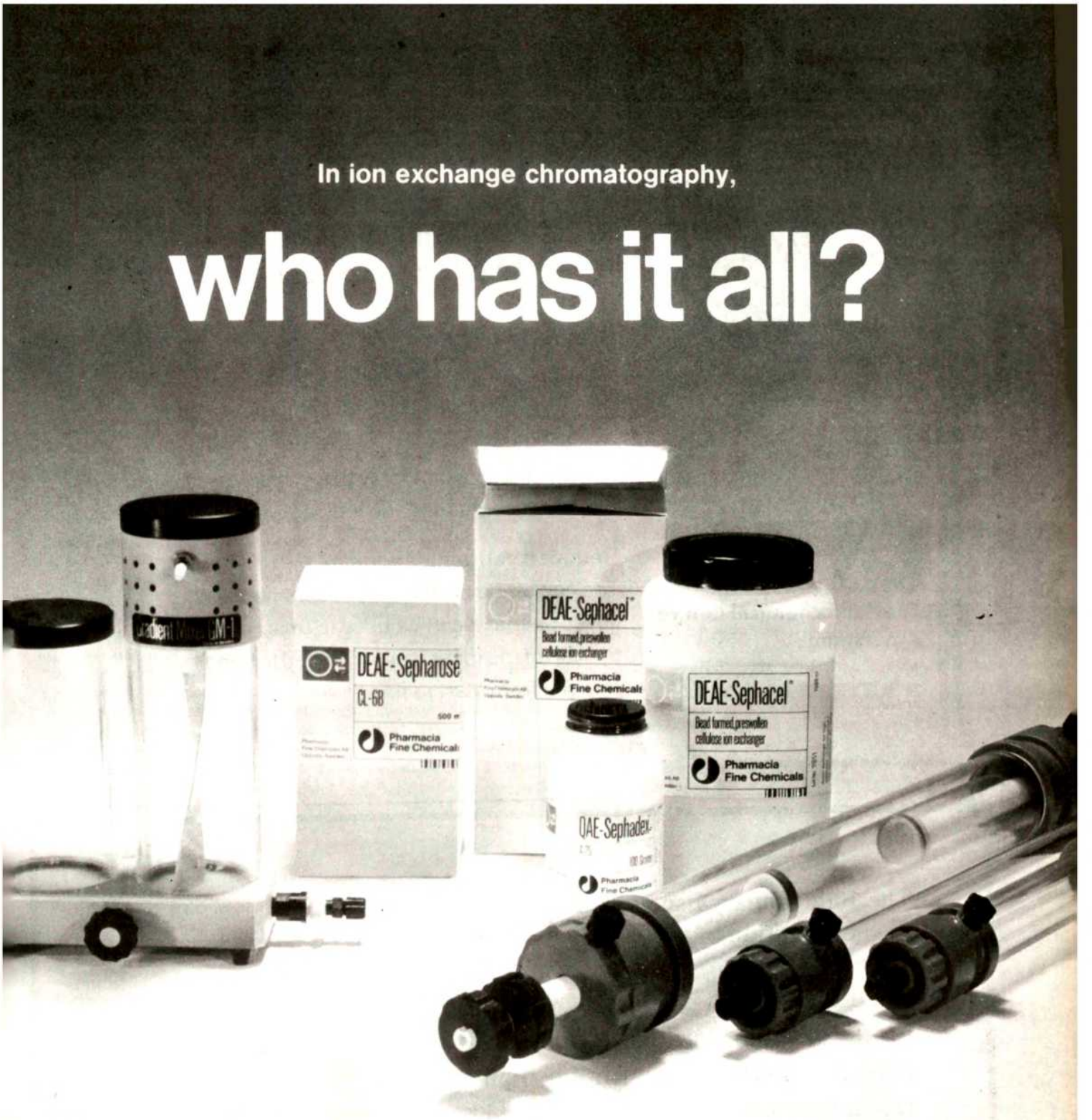
In 1968 he was appointed Professor of Mining at Imperial College and brought back to his old school a wealth of experience in modern mining practice and management. As a teacher, his particular interests were in mineral production management, the design of large open-cast operations and in countering the environmental effects of mining past and future. He was much in demand as a consultant and, as his reputation grew, he became increasingly involved in recalcitrant problems, world-wide, as both technical adviser and arbiter. This heightened authority and experience was used to the benefit of his students — both undergraduate and postgraduate — and of his staff, many of whom were involved with him in the important, topical problems on which he was engaged.

In 1974 he became Head of the Department of Mineral Resources Engineering and, about the same time, began to assume the influence in professional engineering circles that culminated in his presidency of the Institution of Mining and Metallurgy and membership of the Executive Committee of the Council of Engineering Institutions. He did not spare himself, contributing in full to both organizations and serving as chairman and member of a large number of committees. Because he firmly believed that the engineering professions must come closer together, he was also an active Fellow of the Institution of Civil Engineers. He exemplified his conviction by organizing the Tunnelling '76 and Tunnelling '79 International Symposia — highly successful meetings which involved all three Institutions.

With his Presidential Address to the IMM, "Towards a Minerals Policy", delivered in June 1978, Bob Pryor embarked on what was to be his final project — to persuade the professions, industry and government of the need to take active, positive steps to ensure our vital mineral supplies. He followed this up with an informal meeting between government officials and representative mining and metallurgical experts in March 1979, and was one of the prime movers in arranging the National Symposium on the Availability of Strategic Minerals to be held in November of this year. His final contribution was an address entitled "Ensuring Access to Vital Supplies for Britain", which I read to the Parliamentary and Scientific Committee a few days after his death.

There was no badinage, no easy small-talk and certainly no flattery; but to his friends and those who enjoyed his confidence, he was the best of companions — warm and frank, interested and sympathetic, forthcoming and always sincere. Although his own standards were so high, he was in no way smug. He neither expected nor wanted others to share his views except by conviction. He was stubborn but open to reason. He was firm but generous. He had pride, but no conceit and, indeed, he was unduly diffident in some respects. He was humane and tolerant — up to a point — but he could not tolerate dishonesty. If, to these characteristics, you add intelligence, industry, an acute critical faculty, determination and a deep sense of responsibility, you have leadership. If you add courage and absolute integrity, you have a model for a complete professional engineer.

He is survived by his wife Patricia, three sons and a daughter. *M.G. Fleming*



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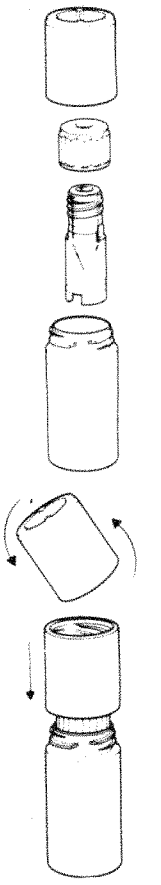
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The 10th January issue of *Nature* will contain extensive coverage in the news section of the rapidly developing field of biotechnology.

Contributions will include:-
Professor Brian Hartley on applications of biotechnology;
Peter Newmark on the scientific origins;
David Dickson on patent issues raised by the new technology;
Robert Walgate on the problems that biotechnology raises for the academic community;
and Sheldon Krimsky and David Baltimore in a debate on the social and political questions.

nature

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announcements

Awards

The Council of the Royal Society has awarded the 1979 Royal Society Esso Award for the Conservation of Energy jointly to **Dr J.E. Harris** and **Dr V.W. Eldred**, for their contribution to the improvement in nuclear fuel utilisation in Magnox nuclear power stations.

Three US investigators, **Drs Solomon Snyder, Max Fink** and **Elliott Gershon**, shared the 1979 Anna Monika Foundation awards for contributions to the understanding of the biological bases of endogenous depression.

The prizewinners of the Feldberg Foundation awards for 1979 are **Dr E. Neher** and **Dr B. Sakmann, MPI** for Biophysical Chemistry, University of Göttingen and **Dr J.L. Gowans**, Secretary of the Medical Research Council and lately Director of the Medical Research Council Cellular Immunology Unit, Oxford.

The European Microwave Conference Prize of 1000 Swiss Francs for the best paper at the Brighton European Microwave Conference, has been won by **Ray Pengelly** of Plessey Research, Caswell.

Biochemical Society Awards: **Dr S.J. Singer** of the Department of Biology, University of California, San Diego, has been invited to deliver the Jubilee Lecture at the Biochemical Society Meeting in December. The Award includes the Harden Medal for 1980 for his distinguished work on the structure and function of membranes. Keilin Medal 1980 has been awarded to **Dr J. Kraut** of the Department of Biochemistry, University of California, San Diego. CIBA Medal and Prize 1979 has been awarded to **Dr J.B. Gurdons, FRS**, of the MRC Laboratory of Molecular Biology, Cambridge. Colworth Medal 1979 has been awarded to **Dr R.A. Laskey** of the MRC Laboratory of Molecular Biology, Cambridge.

Dr Heinz Karger Prize 1979 has been awarded to **Prof. Dr Louis Tobian** (USA). The Dr Heinz Karger Memorial Foundation invites the submission of papers on the following subjects: 1980 — An original research paper on 'Antibodies with Intrinsic Biological Activity'. 1981 — An original research paper on 'Intestinal Absorption of Peptides'. Applications to S Karger AG, Arnold-Böcklin-Strasse 25, CH-4011 Basle, Switzerland.

The 1979 Achievement Award for Scientific Instrument Development has been given to **Dr Harry Freeman**, of Harwell, for his work on laboratory scale heavy ion accelerators for isotope separation and ion implantation.

The British Association's trophy "to the sponsor of the British film released on or since 1 July 1977 which makes the most effective presentation to a non-specialist audience of a scientific or technological subject" was awarded to the University of Manchester/UMIST Audio-Visual Service for *Egyptian Mummies: an Investigation* and it was presented to producer Ken Wrench. Other certificates of merit went to British Airways, *Autoland*; The Central Electricity Generating Board for *Research for Power*; Dr J D Bromhall for *A Clone of Frogs*; and the Shell Company of Hong Kong Ltd for *Four Days in August*.

A new programme of research travel grants for Veterinary Scientists is being launched jointly by The Wellcome Trust of London and the Universities of Guelph and Saskatchewan in Canada.

The scheme is designed to enable Veterinary research workers in Britain/Ireland and Canada to visit each other's country to study, to learn a new research technique, and to work with colleagues with similar interests. The visits are expected to be of relatively short duration, up to three months, and the research grants will meet the costs of travel and subsistence.

Enquiries about visits to Canada from workers in Britain and Ireland should be addressed to The Wellcome Trust, 1 Park Square West, London NW1, UK. Enquiries about visits to Britain and Ireland from workers in Canada should be addressed either to The Dean, College of Graduate Studies and Research, University of Saskatchewan, or to The Dean, Ontario Veterinary College, University of Guelph.

The Directors of the Teylor Foundation and the members of Teyler's Second Society, Haarlem, The Netherlands, announce the following topic for competition for the Teyler Medal: A treatise on the mechanisms which, during morphogenesis, cause the development of genetically determined shapes of parts of organisms (such as, feathery antennae of insects, Grecian nose, and comparable structural parts of plants or micro-organisms). Entries to: The Directors of Teyler's Foundation, Damstraat 21, 2011 HA Haarlem, The Netherlands.

Three hundred National Engineering Scholarships, each worth £500 a year tax free, will be open to high calibre students starting engineering degree courses at universities, polytechnics and other higher education establishments in the academic year 1980–81. The joint Government-industry scholarship scheme is designed to encourage students of high ability to take first degree courses in engineering which tie in with a career in the manufacturing industry. Copies of the pamphlets and application forms are available from Room 7/19, Department of Education and Science, Elizabeth House, York Road, London SE1, UK.

The Advancement of Application of Agriculture and Food Chemistry Award is administered by the Division of Agricultural and Food Chemistry of the ACS and sponsored by International Flavors and Fragrances, Inc. It aims to recognise and encourage outstanding contribution(s) to pure and/or applied agricultural and food chemistry. Nomination forms from the Chairman of the Canvassing Committee, Dr Marshall Phillips, National Animal Disease Center,USDA, PO box 70, Ames, Iowa 50010.

Some hospital posts for junior doctors in Paris and Lyon are reserved specifically for foreigners and will be available from October 1980. Applicants should be at least two years post-registration, under 35 years old and speak fluent French. The posts are of one year duration. Further information from the "Service Scientifique", Ambassade de France, 41 Parkside, Knightsbrige, London SW1, UK.

Under reciprocal agreements with the Centre Nationale de la Recherche Scientifique (CNRS) and the Deutsche Forshungsgemeinschaft (DFG) the Social Science Research Council is able to recommend a number of British candidates for research visits to universities and research establishments in France or the Federal Republic of Germany during 1980/81. Candidacy is limited to social scientists with a working knowledge of French or German on the staff of universities, polytechniques, institutes of further/higher education, and independent research institutes. Further details from SSRC French/German Programmes, Research Initiatives Division, 1 Temple Avenue, London EC4, UK. Quote reference FP7 for France or DP7 for Germany.

Nominations are invited for the 1980 Rosenstiel Award in Oceanographic Science. The award recognises outstanding achievement and distinction in oceanographic science, including relevant aspects of ocean engineering where significant new principles are concerned, and aspects of atmospheric science with important implications for understanding of oceanic processes. The award is supported by the Rosenstiel Foundation through the Rosenstiel School of Marine and Atmospheric Science of the University of Miami. Applications to Dr Richard W Eppley, Institute of Marine Resources, A-018, University of California, San Diego, La Jolla, California 92093.

Meetings

9-10 January, **Medical Applications of Electroanalytical Chemistry,** Newcastle upon Tyne (Dr J.V. Dobson, The Electrochemical Research Laboratories, School of Chemistry, University of Newcastle upon Tyne, UK).

30 January, **Reliability of Semiconductor Components,** London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

30 January, **Geology and Health,** London (Geological Society of London, Burlington House, London W1, UK).

4-7 February, **SYMPHONE,** Berlin CNES, 18 Ave Edouard-Belin, 31055 Toulouse Cedex, France).

17-21 March, **International Conference on Human Herpesviruses,** Atlanta (Tom Sellers, 107 WMCAB, Emory University, Atlanta Georgia 30322).

19 March, **Animal models for the study of Human Nutrition,** London (The Nutrition Society, Chandos House, 2 Queen Anne St, London W1, UK).

9-12 April, **3rd Annual Conference of the Portuguese Chemical Society,** Coimbra (Secretariado do 3°. Encontro Nacional de Química, Departamento de Química, Faculdade de Ciencias e Tecnologia, 300 Coimbra, Portugal).

10-12 April, **Life in the Precambrian,** Leicester (Dr T.D. Ford, Dept of Geology, The University, Leicester, UK).

13-18 April, **Introduction to Toxicology,** Loughborough (Dr G.E. Chivers, University of Technology, Loughborough, Leicestershire, UK).

14-17 April, **Low Energy Ion Beams,** Bath (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

14-18 April, **Postgraduate School on Aspects of Toxicological Testing Methods,** London (R.E. Marshall, School Secretary, Dept of Pharmaceutical Sciences, The Pharmaceutical Society of Great Britain, 1 Lambeth St, London SE1, UK).

14-18 April, **European Rocket and Balloon Programmes,** Bournemouth (Programme Advisory Committee on the ESRANGE Special Project, European Space Agency, 8-10 rue Mario Nikis, 75738 Paris Cedex 15, France).

15-17 April, **Waste-to-Energy,** Cincinnati (Ruth Anne Gibson, Battelle's Columbus Laboratories, 505 King Ave, Columbus, Ohio 43201).

23-30 April, **Remote Sensing of the Environment,** San José (J.J. Cook, Environmental Research Institute of Michigan, PO Box 8618, Ann Arbor, Michigan 48107).

24-25 April, **Grass and Grass Products in the Eighties,** Ayr (Nutrition Society, Chandos House, 2 Queen Anne St, London W1, UK).

25 April, **Aeration of Waste Water,** London (BHRA Fluid Engineering, Cranfield, Bedford, UK).

27 April — 3 May, **EUCHEM Confrence on Stereochemistry,** Burgenstock (Prof. J.D. Dunitz, Laboratorium für Organische Chemie, ETHZ, Universitatsstr. 16, CH-8092 Zürich, Switzerland).

28-30 April, **Economic Effects of Space and other Advanced Technologies,** Strasbourg (European Space Agency, 8-10 rue Mario Nikis, 75738 Paris, Cedex 15, France).

29-30 April, **Programming Microprocessors for Industrial Measurement and Control,** London (Carole Meads, Sira Institute Ltd, Soth Hill, Chislehurst, Kent, UK).

Reports & publications

UK & Ireland — November

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 287, No. 1021: A Marker Induction Mechanism for the Establishment of Ordered Neural Mappings — Its Application to the Retinotectal Problem. By D.J. Willshaw and C. von der Malsburg. Pp. 203-243. (London: The Royal Society, 1979.) UK £3; Overseas £3.20. [511

National Radiological Protection Board. NRPB-R87: The Radiological Consequences of Notional Accidental Releases of Radioactivity from Fast Breeder Reactors: Sensitivity to the Dose-Effect Relationships Adopted for Early Biological Effects. By G.N. Kelly, J.R. Simmonds, H. Smith and J.W. Stather. Pp. 59. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1979. Obtainable from HMSO, London.) £2. [511

The British Library. Sixth Annual Report, 1978-79. Pp. 72. (London: The British Library Store Street, WC1, 1979.) [511

Francis Galton, A.J. Balfour and Eugenics. By A. Armstrong. Pp. 7. 35p. Mind and Muscle, 9,000 B.C. — 2,000 A.D. By A. Armstrong. Pp. 10. 35pp. Mind and Muscle — 2, Carlyle, Marx, and Galton. By A. Armstrong. Pp. 11. 35p. (Hatfield, Herts.: The HiSci Press, c/o 4a Harpsfield Broadway, 1979.) [611

Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 79, No. 5: Interpolation and Approximation of Entire Functions. By O.P. Juneja and S.R.H. Rizvi. Pp. 37-47. £1.70. Vol. 79, No. 8: An Automatic Photoelectric Condensation Nucleus Counter with Digital Recording. By G.G. McMahon and J.A. Scott. Pp. 73-£80. £1. Vol. 79, No. 9: A Theorem on Entire Harmonic Functions of Zero Exponential Type. Pp. 81-85. 90p. Vol. 79, No. 10: The Endomorphism Near-Ring of Finite General Linear Groups. By J.D.P. Meldrum. Pp.87-96. £1.20. Vol. 79, No. 11: Rational Approximation in Lipschitz Norms — II. By A.G. O'Farrell. Pp. 103-114. £1.50. Vol. 79, No. 13: *O*-Primitice Near-Rings of Transformations. By D. Ramakotaiah and G.K. Rao. Pp. 131-146. £1.95. Section B. Biological, Geological and Chemical Science. Vol. 79, No. 13: On the Sites of Interaction of Oxidizing Substrates with Molybdenum Iron/Sulphut Flavin Hydroxylases. By M.P. Coughland and Ide Ni Fhaolain. Pp. 169-175. Vol. 79, No. 79, No. 14: Petrogenesis of the Contact Rocks, Blind Rock Dyke, County Donegal. By R. Nawaz. Pp. 177-189 £1.20. Vol. 79 No. 15: The Geology of the Northern Part of the Murrisk Trough. By T.J. Kelly and M.D. Max. Pp. 191-206. £1.60. (Dublin: Royal Irish Academy, 1979.) [1911

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences, Vol. 292, No. 1398: Proton-Hydrogen Collisions: Differential and Total Cross Sections for H(2s) and H (2p) Production in the 1-7- kev Range. By D.S.F. Crothers and J.G. Hughes. Pp. 539-561. UK £1.65; Overseas £2. Vol. 292, No. 1399: The Syrface Structure and Composition of Layered Silicate Minerals — Novel Insights from X-ray Photoelectron Diffraction, K-emission Spectroscopy and Cognate Techniqques. By S. Evans, J.M. Adams and J.M. Thomas, F.R.S. Pp. 563-591. UK £2.10; Overseas £2.45.

The British Council. Annual Report, 1978-79. Pp.59. 'London: The British Council, 1979.) [1911

Annual Report on the Meteorological Office, 1978. (Met. O. 928). Pp.xiv + 158. (London: HMSO, 1979.) £4. net. [1911

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Technical Report No. 51: The Field Assessment of Effects of Dumping Wastes at Sea. 5: The Disposal of Solid Wastes Off the North-East Coast of England. By R.A. Eagle, P.A. Hardiman, M.G. Norton, R.S. Nunny and M.S. Rolfe. Pp.34. Technical Report No. 52: The Composition of Dredged Spoils Dumped at Sea from England and Wales. By Lindsay A. Murray and M.G. Norton. Pp. 10. (Lowestoft: Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, 1979.) [1911

A Key to the British Freshwater Leeches, with Notes on Their Life Cycles and Ecology. By J.M. Elliott and K.H. Mann. (Scientific Publication No. 40.) Pp. 72. £2. A Guide to Methods for Estimating Microbial Numbers and Biomass in Fresh Water. By J.C. Jones. (Scientific Publication No. 39). Pp. 112. £2.50. (Ambleside, Cumbria: Freshwater Biological Association, 1979.)

Potato Marketing Board. Annual Report and Accounts, 1979. Pp. 52. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1979.) [2011

Office of Population Censuses and Surveys. Mortality Statistics, 1977, England and Wales. (Series DH1 No. 5.) Pp. xiv + 105. (London: HMSO, 1979.) £4.25 net. [2011

Other countries — November

Physics at High Energies. (Proceedings of the Stockholm University Centennial Symposium devoted to the Quark Concept, held in Stockholm in September 1978.) Edited by G. Ekspong and S.P. Holmgren. (Reprinted from *Physica Scripta*, Vol. 20, 1979.) Pp. 211-288. (Stockholm: The Royal Swedish Academy of Sciences, 1979.) Sw.kr.100. [111

The Effects of the Dioxins on the Environment: a Bibliography. (Library Bibliography Series, No. 7.) Pp.13. (Orlando, Florida: Gifts and Exchange Department, University of Central Florida Libraries, PO Box 25000, 1979.) $1. [211

Proceedings of R.O.C.-United States Cooperative Science Seminar on Mycoplasma Diseases in Plants. Edited by Hong-Ji Su and Randolph E. McCoy. (NSC Symposium Series, No. 1.) Pp.xv + 179. (Taipei, Taiwan, Republic of China: National Science Council, 1979.) [211

European Molecular Biology Laboratory. Research Reports 1978. Pp. 217 (49 plates). (Heidelberg: EMBL, 1979.) [211

Smithsonian Contributions to Zoology, No. 295: Studies of Ephydrinae (Diptera: Ephydridae), II: Phylogeny, Classification, and Zoogeography of Nearctic *Lamproscatella* Hendel. By Wayne N. Mathis. Pp. iii + 41. (Washington, DC: Smithsonian Institution Press, 1979. For sale by US Government Printing Office.) [211

Annals of the South African Museum. Vol. 79, Part 4: The South African Museum's *Meiring Naude* Cruises, Part 9: Bryozoa. By P.J. Hayward and P.L. Cooke. Pp.43-130. Vol. 79, Part 5: A Redescription of the Kelp Curler *Ampithoe humeralis* (Crustacea, Amphipoda) from South Africa and Its Relationship to *Macropisthopous*. By Charles L. Griffiths. Pp. 131-138. (Cape Town: South African Museum, 1979.) [511

Republic of Cyprus. Ministry of Agriculture and Natural Resources. Annual Report of the Geological Survey Department for the Year 1978. By Th. M. Pantazis. Pp. 59. (Nicosia, Cyprus: Geological Survey Department, 1979.) [2011

United States Department of the Interior: Geological Survey Department. Porofessional Paper 1083: Environmental Implications of Element Emissions from Phosphate-Processing Operations in Southeastern Idaho. By R.C. Severson and L.P. Gough. Pp. iii + 20. (Washington, DC:. US Government Printing Office, 1979.) [2011

Unesco. Bruun Memorial Lectures — The Importance and Application of Satellite and Remotely Sensed Data to Oceanography. (Intergovernmental Oceanographic Commission Technical Series, No. 19.) Pp. 64. (Paris: Unesco, 1979.) [2011

Science Council of Canada. Annual Report, 1978-79. (No. 13). Pp. 85. (Ottawa: Science Council of Canada, 1979.) [2011

Scientific Research in Israel, 1979. Pp. 299. (Jerusalem: Ministry of Energy and Infrastructure, National Council for Research and Development, 1979.) [2011

Bulletin of the American Museum of Natural History. Vol. 164, Article 3: Ornithological Literature of the Papuan Subregion 1915 to 1976 — An Annotated Bibliography. By Clifford B. Frith. Pp. 377-465. (New York: American Museum of Natural History, 1979.) $6.15. [2011

Centre National pour l'Exploitation des Oceans. Actes de Colloques No. 9: Les Côtes Atlantiques de l'Europe — Evolution, Amébagement, Protection, Brest, 15-16 Mai, 1979. Préparé par Pr. André Guilcher. Pp. 301. (Brest Cedex: Section Documentation, Centre Océanologique de Bretagne, 1979.)

newly on the market

These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. For more details use the Reader Enquiry Card bound inside the journal.

X-Ray fluorescence analyser. Jobin-Yvon. The Model JY 410 Portarix from Jobin-Yvon is a portable radio isotope X-ray fluorescence analyser designed for elemental analysis of ores, steel alloys, cement and other materials, identification of foreign substances in materials and thickness measurements of coated metals both in industry and laboratories. The Portarix is a compact, light-weight and robust instrument which can be carried by one man from place to place in the field.
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Nucleonics Accessories brochure. Packard announce a new brochure giving details on their extensive range of scintillation cocktails, reagents, vials and reference materials for liquid scintillation and automatic γ-counting techniques. The brochure contains details on over 250 separate items of scintillation counting consumables.
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γ-Counters. The Philips PW 4800 series of γ-counters combine latest state-of-the-art technology with maximum reliability and minimum cost to the user. Due to the incorporation of microprocessors, performance is of a very high order. Standard features include background subtract and automatic compensation for long-term photomultiplier gain drift, using a background standard. The extent of amplification adjustment is given, with an indication if beyond set limits. Data are produced as c.p.m., % c.p.m., normalised c.p.m., and, for radioimmunoassay, % bound. With the optional integral calculator, conversion of count into disintegrations per min is automatic, after calibration with a standard. For dual label programs a similar procedure gives automatic correction for spillover between low and high energy windows.
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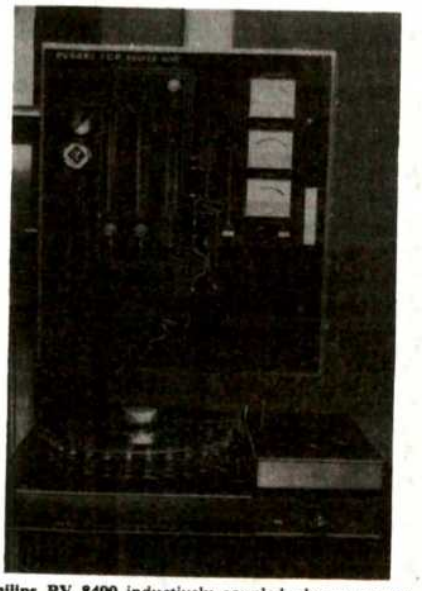
Flashlamp system. Edinburgh Instruments. The EI-199F coaxial flashlamp system is a versatile pulsed light source designed for a wide range of applications including fluorescence, phosphorescence, photochemistry, laser simulation and fibre optic cable and detector testing. The complete system includes the thyratron gated flashlamp, high voltage power supply and triggering unit, and monitoring photomultiplier and power supply. Variable pulse widths to below 1 ns at continuously tunable repetition rates from 1 Hz to 100 Hz can be obtained. Stability monitoring of every light pulse is included, and the flashlamp's all-metal coaxial construction eliminates rf interference problems.
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Liquid scintillation counters. The Philips liquid scintillation counters, series PW 4700, have extensive measurement correction and data processing abilities, improved reliability and reduced prices. Use of microprocessors provides four measurement correction functions, which together with a random coincidence monitor, give 10 combinations. The extent of quenching for single labelled samples can be measured using either the sample channels ratio or by the external standard channels ratio. The latter method is applicable to both single and dual label samples.
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Energy-dispersive X-ray spectrometry system. Philips. Fast, simultaneous multi-element analysis capability at modest cost, without the limitation of a fixed number of measuring channels, is possible using the Philips PV 9500 energy-dispersive X-ray spectrometry system. The system permits identification and determination of all elements from sodium to uranium in solids, powders and liquids. Measurements are possible from major constituent levels down to a few p.p.m. Addition of a specially developed spectrometer unit to the recently introduced PV 9100 multi-channel analyser has produced a compact, self-contained system that is easily installed in any location. The caster-mounted spectrometer and analyser consoles can be independently sited to suit available space. No mains services other than electrical connection are required.
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Workgloves. DuPont. Parabest 'Premier' gloves from DuPont are made from Kevlar 29 fabric, a material with outstanding properties of cut, puncture and heat resistance. In addition, they offer excellent thermal insulation, and so can be used safely and comfortably in higher heat conditions and for longer working periods than is possible with leather or asbestos.
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Polarographic stand. Bruker. The Model E-2 polarographic stand from Bruker eliminates the classical problem of polarography where too many wires, tubes and electrodes have to be brought into a small cell. In this new design of stand, electrical and gas connections are short and tidy; the mercury or glassy carbon working electrode is easily inserted or removed and a stirrer is incorporated. The result is a tidy and convenient experimental set up.
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HARRIS, Stephen. Secret Life of the Harvest Mouse. Photographs Oxford Scientific Films Limited. Pp.77. ISBN-0-600-34582-3. (London, New York, Sydney and Toronto: Hamlyn, 1979.) £2.95.

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HUGHES, David. The Star of Bethlehem Mystery. Pp.xiii + 218. ISBN-0-460-04343-9. (London, Toronto and Melbourne: J.M. Dent and Sons, Ltd., 1979.) £7.95 net.

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MARGERSION, Tom, WALLACE, Marjorie and HALLENSTEIN, Dalbert. The Superpoison. Pp.236. ISBN-0-333-22797-2. (London and Basingstoke: Macmillan London, Ltd., 1979.) £7.95 net.

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SCHACHTER, Frances Fuchs, *et al.* Everyday Mother Talk to toddlers: Early Intervention. Pp.xvii + 211. ISBN-0-12-621360-7. (New York and London: Academic Press, Publishers, 1979.) $14.50.

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SHOWERS, Victor. World Facts and Figures: a Unique, Authoritative Collection of Comparative Information About Cities, Countries, and Geographic Features of the World. Pp.xii + 757. ISBN-0-471-04941-7. (New York and chichester: Wiley-Interscience, John Wiley and Sons, 1979.) £11.75.

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TUNNICLIFFE, C.F. A Sketchbook of Birds. Pp.123. ISBN-0-575-02640-5. (London: Victor Gollanez, Ltd., 1979.) £7.95 net.

WARD, Peter. Colour for Survival. Pp.120. ISBN-0-85613-093-1. (London: Orbis Publishing, 1979.) £6.95.

WARD-HARRIS, Joan. Creature Comforts. Pp.204 + 17 photographs. ISBN-0-00-262119-3. (London: Collins and Harvill Press, 1979.) £5.95.

WITTGENSTEIN, Ludwig. Remarks on Colour. Ed. by G.E.M. Anscombe. Translated by Linda L. McAlister and Margarete Schattle. Pp.63. ISBN-0-631-11641-9. (Oxford: Basil Blackwell, 1979.) £2.95.

Anthropology

BLACKBURN, Julia. The White Men: The First Response of aboriginal peoples to the White Man. Pp.192. ISBN-0-85613-399-X. (London: Orbis Publishing, Ltd., 1979.) £6.75 net.

CHAGNON, Napoleon A., and IRONS, William (ed.). Evolutionary Biology and Human Social Behavior: An Anthropological Perspective. Pp.xvi + 623. ISBN-0-87872-193-2. (North Scituate, Mass.: Duxbury Press, a Division of Wadsworth, Inc., 1979.)

LEAKEY, Mary D. Olduvai Gorge: My Search for Early Man. Pp.187 + 11 plates. ISBN-0-00-211613-8. (London and Glasgow: William Collins Sons and Co., Ltd., 1979.) £6.50 net. (Nov.)

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APPOINTMENTS VACANT

FACULTY POSITIONS

he Department of Zoology at the niversity of Texas at Austin is recruiting o fill two vacancies.

1) GENETICIST

hose research deals with the eucaryotic enome and its structure and/or function. his opening is at the senior level. The areas f greatest interest are: somatic cell, mole- ular and/or developmental genetics.

2) REPRODUCTIVE PHYSIOLOGIST

hose research is on the vertebrate central ervous system investigated anatomically nd/or biochemically. This individual ould be able to relate neural processes to production with an emphasis on the rmer. This position is open at the ssistant professor level, however, senior pplicants will be considered.

Applicants should submit: a curriculum tae, selected reprints, a brief statement of search plans, qualifications for graduate d undergraduate instruction, and names f 5 references.

Applications should be sent by ecember 1 1979 to the Chairman, epartment of Zoology, University of exas at Austin, Austin, Texas 78712.

The University of Texas at Austin is an qual Opportunity/Affirmative Action mployer. W609(A)

DIRECTOR, NTERNATIONAL CENTRE FOR DIARRHOEAL DISEASES RESEARCH, BANGLADESH (ICDDR, B)

he ICDDR, B is an international health search and training centre located in angladesh, and is directed toward the udy of all aspects of diarrhoeal diseases nd related aspects of fertility and utrition. It was established by an rdinance of the Government of angladesh in December, 1978. Twenty-six ations and agencies now participate in the ork of the centre. It is guided by a Board f Trustees with majority representation om developing countries. The director is esponsible for the programs and anagement of the centre.

A candidate should have a strong inter- ational reputation as a biomedical cientist and experience in managing a cientific organization. Experience in eveloping countries is desirable.

The terms and conditions of mployment are negotiable, and may be uided by policies of other international nstitutions.

Letters of application should contain a omplete curriculum vitae and should be ddressed to: Charles C.J. Carpenter, MD, hairman, Department of Medicine, Case Vestern Reserve University, Cleveland, hio 44106. W633(A)

DIRECTOR, LABORATORY FOR PLASMA AND FUSION ENERGY STUDIES

The University of Maryland at College Park invites applications and nominations for the position of Director of the Laboratory for Plasma and Fusion Energy Studies. Candidates for the position should have a distinguished reputation in research, the ability to lead in further developing the programs of a newly established laboratory and be highly qualified for appointment as a tenured Professor in a relevant department of physical science or engineering.

The prospective appointment date is on or about July 1, 1980; applications received by March 1, 1980, will receive full consideration.

Inquiries, nominations, and applications should be addressed to Dr Robert O Harger, Chairman of the Search Committee, Electrical Engineering Department, University of Maryland, College Park, Maryland, 20742. The University of Maryland is an equal opportunity, affirmative action employer. W629(A)

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF UROLOGY

SCIENTIST

Full-time faculty position in Urology Department for basic scientist. We seek a person able to initiate and conduct his/her own research programs, supervise research residents and fellows, and participate in teaching of undergraduate, graduate and medical students in selected areas of immunology. Minimum criteria are PhD and/or MD degree with demonstrated competence in conducting independent research in immunology. The UC Davis campus offers unusual opportunities in basic immunologic research because of immediate proximity to the Medical School, Veterinary School and Primate Centre. A joint appointment with the appropriate Basic Science Department will be considered. Send curiculum vitae to John M. Palmer, MD, Chair, Department of Urology, UC Davis School of Medicine, 4301 X Street, Suite 249, Sacramento, CA 95817. In compliance with federal and state laws and University policy, the University of California does not discriminate on the basis of race, colour, national origin, religion, sex, handicap, age or against disabled veterans or veterans of the Vietnam era. The University is an Affirmative Action/Equal Opportunity Employer. Position open until filled. W642(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH POST

Postdoctoral applicants with experience in materials science, physics or related subjects for a 3 year research post funded by the Arthritis and Rheumatism Council to investigate mechanical properties and morphology of collageneous tissues. Previous experience in biomechanics, electron microscopy or related areas and interest in medically orientated physics or engineering is desirable. Salary in the range £4333 to £5488. (Scale under review).

Applications, including names of two referees and curriculum vitae should be sent to Dr J Shah, Department of Physics, University of Bristol, Bristol BS8 1TL by 15th February, 1980. 3974(A)

UNIVERSITY OF LONDON (BOARD OF STUDIES IN ZOOLOGY)

STEREOSCAN UNIT AT BEDFORD COLLEGE

SEM TECHNICIAN GRADE 5

required to be responsible for the operation and maintenance of the Stereoscan Microscope Unit. Duties will include preparative work associated with operation of the equipment.

Gross salary range (under review) £4480 to £5100.

Application forms from Personnel Office, Bedford College, Regent's Park, London, NW1 4NS (tel: 01-486 4400 Ext. 312). 3962(A)

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The College is situated 50km north of Adelaide, South Australia on a 1200 hectare farming property. Tertiary courses are offered in Natural Resources, Agriculture, Oenology, Wine Marketing and Farm Management.

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DUTIES: To provide professional leadership in this field. To present a series of subjects centred on the legal, social and political background to the use of Natural Resources. The appointee will be involved in subjects such as Natural Resource Legislation, Environmental Impact Statement Methodology, Environmental Indices and Monitoring, Management Plants, Quantification of Soil, Water Vegetation and Animal Communities. A knowledge of cartographic and remote sensing techniques would be desirable.

QUALIFICATIONS AND EXPERIENCE: A degree in Science, Ag. Science or related area, with post-graduate qualifications. Involvement in research or experience in Industry or Government service at a Senior level regarded as essential.

SALARY RANGES: **Principal Lecturer up to $28,813**
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Senior Lecturer II $21,861 to $23,408

APPLICATIONS: including name, address, date of birth, telephone number and qualifications, along with names and addresses of 3 referees, should be marked "Confidential" and reach the Administrative Secretary, Roseworthy Agricultural College, Roseworthy, South Australia, 5371 by January 11th 1980. W630(A)

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IMPERIAL CHEMICAL INDUSTRIES LTD

UNIVERSITY OF EDINBURGH UNIVERSITY OF ABERDEEN

THE SYNTHESIS, STRUCTURE AND PROPERTIES OF NOVEL ZEOLITES

Applications are invited for the following positions financed by a Cooperative Award from the Science Research Council and ICI Ltd.

These posts offer a rare opportunity to participate in a large collaborative investigation into the synthesis and properties of an important new generation of materials which show great promise as sorbents and catalysts.

1. A Postdoctoral Research Associate to work with Drs B M Lowe and H F Leach at the University of Edinburgh on the hydrothermal synthesis and characterisation (by X-ray powder diffraction, thermal analysis and sorption) of Novel Zeolites.

2. A Postdoctoral Research Associate to work with Dr J A Gard at the University of Aberdeen on the determination of the structure of zeolites by X-ray diffraction, electron diffraction and related techniques.

3. A Postdoctoral Research Associate to work with Dr D A Whan at the University of Edinburgh on catalysis by zeolites. The principal techniques involved will be gas chromatography and mass spectrometry.

4. A Technician to work with Dr B M Lowe at the University of Edinburgh on the synthesis of novel zeolites.

5. A Technician to work with Drs H F Leach and D A Whan at the University of Edinburgh on the adsorptive and catalytic properties of zeolites.

Previous experience in the appropriate field would be advantageous but is not essential.

Appointments, which will commence between March and October 1980, will be for a period of two years with the possibility of renewal for a further period.

Initial salaries for Postdoctoral Research Associates will be in the range £4,622 to £5,488 (under review) on scale 1A for Research and Analogous Staff.

Initial salaries for Technicians will be in the range £3,699 to £4,065 (under review) on scale TG5. Further information about specific positions may be obtained directly from the academic staff members indicated.

Applications (3 copies), including curriculum vitae and the names and addresses of two referees should be sent as soon as possible and not later than 1st Jan. to

Dr N H Ray,
The Corporate Laboratory,
Imperial Chemical Industries Ltd,
P.O. Box No. 11,
The Heath, Runcorn, Cheshire WA7 4QE 3992(A)

STAFF FOR THE BASIC SCIENCES UNIVERSITY OF IOANNINA MEDICAL SCHOOL

The University of Ioannina Medical School has openings for positions of EPIMELITAI starting March 1980 or later in the following Laboratories: 1) Biological Chemistry; 2) Biology; 3) Medical Physics; 4) Microbiology; 5) Pharmacology; 6) Physiology.

Candidates should be fluent in Greek, have a PhD in the above or related fields, or an MD with strong interests and experience in the above subjects.

The successful candidates will be expected to take part in the teaching programme for about 100 medical students and participate in research in the above areas. The basic salary is about 20,000Drs per month before taxes and is expected to be supplemented from other sources.

Applicants should forward their curriculum vitae with bibliography, two or more letters of recommendation, and a brief outline of research interests to: Orestes Tsolas, PhD Professor, Laboratory of Biological Chemistry, University of Ioannina Medical School, Ioannina Greece (Telephone 0651 33442). W634(A)

UNIVERSITY OF QUEENSLAND

LECTURER IN PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

The appointee will be expected to pursue an active research programme. Present areas of research activity include female reproduction, endocrinology, fetal and cardiovascular — renal physiology and andrology. 29.2.80.

SENIOR TUTOR IN BIOCHEMISTRY

Minimum qualification is a PhD in Biochemistry. The appointee will be required to undertake teaching and research in Biochemistry. 4.1.80.

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Applicants should have a higher degree or Honours and experience in Vertebrate Zoology. Ability to organise practical classes essential. 7.1.80.

Salaries: Lecturer: $A16,291 to $A21,401 p.a. Senior Tutor: $A14,041 to $A16,085 p.a.

Other benefits: Lecturer: Opportunities to undertake programme of special studies, superannuation, housing assistance, travelling and removal expenses. Snr. Tutor: Superannuation, housing assistance, travelling and removal expenses.

Additional information and application forms are obtainable from the Association of Commonwealth Universities, (Appts) 36 Gordon Square, London WC1H 0PF.

ARGC RESEARCH ASSISTANT GRADE 1 PHYSIOLOGY & PHARMACOLOGY

A post-doctoral graduate with biochemical experience required for reproductive endocrinology on the role of granules and binding proteins in the secretion of steroid hormones. Experience in one of the following areas would be an advantage: steroid biochemistry, protein purification, sub-cellular fractionation. Enquiries: Professor G.D. Thorburn, University of Queensland, St. Lucia, Queensland 4067, Australia. Quote Ref No 44979. 3987(A)

UNIVERSITY OF MARBURG

DEPARTMENT OF ANATOMY AND CELL BIOLOGY

RESEARCH AND TEACHING ASSISTANT

Applications are invited for a postgraduate research and teaching assistant, who is required to work on developmental aspects of the autonomic nervous system.

Experience in neurobiology, particularly in neurochemistry, cell culture or electron microscopy would be an advantage.

Salary according to BAT IIa (DM3,000 to DM3,200 per month).

The appointment may be taken up immediately.

Applications (two copies) giving details of age, education, language abilities and experience together with the names and addresses of two referees should be sent to Professor K. Unsicker, Department of Anatomy and Cell Biology, Philipps-University, D-3550 Marburg, West Germany. W638(A)

FACULTY POSITION

DEPARTMENT OF BIOLOGICAL CHEMISTRY

UNIVERSITY OF CALIFORNIA

Davis

School of Medicine

Applications are invited for a faculty position at the rank of assistant or associate professor, to begin Spring or Summer 1980.

Candidate should be active in research capable of contributing significantly to Medical School and Graduate teaching in the areas of biochemistry, physiological chemistry, cellular and molecular biology.

Quality and originality of research will receive greater emphasis than field of specialization although potential to enter into interdisciplinary research within the Department, encompassing the areas mentioned above will be given favorable consideration.

Applicants should send a curriculum vitae, list of publications, future research plans and the names of at least three individuals from whom letters of recommendation may be solicited by the Search Committee to: Dr. Robert R. Traut Chairperson, Search Committee Department of Biological Chemistry School of Medicine, University of California, Davis, CA 95616.

Completed applications should be received not later than January 31, 1980. The University of California is an affirmative action/equal opportunity employer. Women and minority candidates are encouraged to apply. W636(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GEOGRAPHY

Applications are invited for the post of

RESEARCH ASSISTANT

to assist Dr A Henderson-Sellers in NERC funded theoretical investigation of the methods of parameterising albedo using satellite data. Candidates should have a good science degree including considerable experience in computing techniques. Some knowledge of atmospheric physics would be useful.

The post is tenable for one year at a salary of £3,775 per annum.

Applications, together with the names of three referees, should be received not later than 18th January, 1980 by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/873. 3984(A)

GUY'S HOSPITAL

MEDICAL SCHOOL

POSTDOCTORAL RESEARCH ASSISTANT

required to work with Dr L Mallucci in the Department of Microbiology on a project supported by the Cancer Research Campaign on "The Role of Surface Components in Control of Cell Growth".

Research will involve isolation and characterisation of components that affect events related to cell division.

The appointment is available for three years and the initial salary will be within the range £4,033 to £6,066 (under revision) plus £740 London Allowance.

Apply in writing with full curriculum vitae and the names of two referees to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C. 3993(A)

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Entomologist/ Insecticides Research

Shell Biosciences Laboratory has a vacancy for a graduate entomologist to work in the Biological Evaluation Division of their Sittingbourne Research Centre in Kent. The person appointed will become a member of an innovative and lively team involved in the search for new insecticides. A basic knowledge of and interest in entomology including insect rearing techniques is considered essential, together with sufficient knowledge of organic chemistry to appreciate the structure/activity relationship. The post is seen as an important and challenging one at a time when the future course of insect pest control is being widely reviewed. The job will be laboratory based primarily although opportunities for field work will arise.

Sittingbourne Research Centre is a large, modern, well-equipped site with outstanding Laboratory, sports and social facilities and an excellent subsidised staff restaurant. We offer a competitive salary and good career prospects.

Applicants should be below 30 years of age, have at least an upper second class honours degree, and post-graduate experience in entomology preferably leading to a PhD.

Please write or telephone for an application form to:

Shell Research Limited, The Recruitment Division, (N1) PNEL/11, Shell Centre, London. Telephone 01-934 2948.

Shell

3972(A)

THE UNIVERSITY OF HULL

LECTURER IN GEOPHYSICS

pplications are invited for the post of ecturer in Geophysics in the Department f Geology, tenable from 1 October, 1980. he person appointed will be responsible or teaching courses in geophysics, xploration geophysics and geological map ork. Preference may be given to ndidates with research interests in the ophysics of mineral exploration.

Salary will be on the scale £4333 to £8992 er annum (under review) with initial acing in the range £4333 to £5199 per nnum.

Applications (six copies) should give full articulars of age, qualifications and xperience, together with the names of ree persons from whom references can be ought, and be lodged not later than 1 larch 1980, with The Registrar, University f Hull, HU6 7RX, from whom further articulars can be obtained. 3975(A)

UNIVERSITY OF ST. ANDREWS

DEPARTMENT OF PSYCHOLOGY

Applications are invited for the post of RESEARCH ASSISTANT in the Department of Psychology, primarily to work on a three-year S.R.C. sponsored project on The Role of the Superior Colliculus in Pattern Perception in Rats.

Applications should preferably have a degree in Psychology, but graduates in related disciplines who have a strong interest in physiological psychology will be considered.

Starting salary £3,775 per annum on Research scale 1B, plus U.S.S.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by *31st January 1980* with the Establishments Officer, The University, College Gate, St. Andrews, Fife, from whom further particulars may be obtained. 3990(A)

RUHR-UNIVERSITÄT BOCHUM

An der **Abteilung für Biologie** ist die Stelle eines

Ordentlichen Professors (H4) für Pflanzenphysiologie

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Bewerbungen mit Lebenslauf und Schriftenverzeichnis sind bis zum **31.3.1980** and den **Dekan der Abteilung für Biologie, Postfach 10 21 48, 4630 Bochum 1,** zu richten. W628(A)

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Positions in
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Boston, Massachusetts

Tufts University, School of Medicine, an academic institution actively involved in research, has two positions available in the field of Electron Microscopy. We are seeking skilled, enthusiastic applicants who have a commitment to continued self development. Both of these jobs entail working closely with several investigators and our needs are best filled by a congenial, highly motivated person.

MOLECULAR BIOLOGY

Individual will have prime responsibility for youth, operational maintenance and supervision of a multi-user EM complex which includes a JOEL 100S and 100B. This person will serve as a resource, helping investigators choose, develop, learn and apply EM techniques to their particular research problems. Applicants must have some experience in negative staining, evaporation, and appropriate darkroom techniques. Many of the applications involve spreading and visualizing molecular structures such as DNA heteroduplexes, nucleic acid-protein complexes and phage substructure.

ANATOMY

This department requires the cultivated skills of a person to supervise a common access facility for transmission electron microscopy. Candidates should demonstrate experience in: (1) tissue preparation, embedding, sectioning and contrast staining of epoxy embedded tissue. (2) Operator maintenance of EM including filamenting and stigmation of electron microscopes (Phillips AM200, Joelco 100B, RCA EMU-3H) and (3) general photography, particularly darkroom work. The individual will be expected both to help department personnel learn instrument use and procedures and also to perform technical work for some of the faculty involved.

To apply for either of the above positions, please submit résumé, specifying which one interests you. Please include your salary history and requirements and send to: Tufts University, Personnel Office, Attention: Ina-Lee Block, 63 Kneeland Street, Boston, MA 02111 USA.

An Equal Opportunity/Affirmative Action Employer

W637(A)

TUFTS university

HOUGHTON POULTRY RESEARCH STATION

TEMPORARY SENIOR SCIENTIFIC OFFICER AND HIGHER SCIENTIFIC OFFICER IN THE PARASITOLOGY DEPARTMENT

Vacancies exist for a Senior Scientific Officer and a Higher Scientific Officer in a team investigating the production of attenuated strains of the species of *Eimeria* causing coccidiosis in domestic poultry, and their utilisation for immunisation of young chickens. The appointments are temporary for three years funded by the National Research Development Corporation as part of a programme of research to develop an effective vaccine against coccidiosis.

SENIOR SCIENTIFIC OFFICER

Applicants should have at least four years post-graduate research or other approved experience, and should hold a PhD degree with post-doctoral experience in protozoan biology, cell culture or biochemistry.

HIGHER SCIENTIFIC OFFICER

The post is of a technical nature and applicants should have a degree, HNC or equivalent in a science subject, with at least 5 years relevant post qualifying experience, with appropriate practical experience in microbiology. Experience in protozoology is not necessary.

Appointment in the Senior Scientific Officer grade will be on a salary within the scale effective from 1st January 1980, £6,332 to £6,957, and the appointment in the Higher Scientific Officer grade will be on a salary within the scale effective from 1st January 1980, £5,097 to £6,030. Non-contributory superannuation scheme.

Further particulars and application form from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA. Closing date for applications 25th January, 1980. 3973(A)

ROYAL UNITED HOSPITAL, BATH

RESEARCH BIOCHEMIST

Required to take part in a 3 year study of platelet and lipid metabolism in diabetic subjects. Post based in the Laboratories of the Clinical Investigation Dept and facilities also available in the Dept of Biochemistry at Bath University. Salary at the appropriate point on the scale for Hospital Biochemists.

Enquiries to Dr. J.P.D. Reckless on Bath 28331 Ext 735.

Application forms from Sector Administrator, Royal United Hospital, Bath. Tel: Bath 28331 Ext 606. 3971(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF ZOOLOGY

POSTGRADUATE RESEARCH ASSISTANT

Applications are invited from graduates with interests in Cell Biology and Neurobiology to work on a project investigating the appearance and distribution of glutamate receptors on locust muscle. Techniques involved will include electron microscopy and freeze-etching and some practical experience in neurobiology or electron microscopy would be an advantage.

The project is funded by SRC for three years and the appointment will be made as soon as possible at a starting salary of £3,775. Applicants may register for a higher degree if they wish to.

Letters of application including the names of two referees to Dr I R Duce, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD. 3986(A)

THE ROYAL VETERINARY COLLEGE
University of London

DIVISION OF PARACLINICAL STUDIES

DEPARTMENT OF PATHOLOGY

LECTURER IN VETERINARY PATHOLOGY

Applications are invited from veterinary graduates for the post of Lecturer in Veterinary Pathology. Duties will include diagnostic work and participation in teaching the undergraduate and postgraduate courses in pathology. The successful applicant will be encouraged to take advantage of the good research opportunities which are available.

Preference will be given to applicants having some relevant experience in post-mortem diagnosis, histopathology or similar fields, but consideration will be given to applications from recent graduates or others.

Salary scale (under review): £5073 to £9732 inclusive of London Allowance. Initial salary will be determined according to qualifications and experience. Superannuation under the Universities' scheme.

Removal expenses up to £800 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone: 01-387 2898.

Closing date for applications: 14 January 1980. 3963(A)

UNIVERSITY OF SOUTHAMPTON

CRC MEDICAL ONCOLOGY UNI

Applications are invited for t position of

POST-DOCTORAL RESEARCH ASSISTANT

to work on the isolation and purificatio membrane proteins and glycoprot involved in cell adhesion of *Dictyostel discoideum*. Candidates should have, o about to obtain, a PhD in Biochemis Some experience of working with m branes, proteins or carbohydrates woul advantageous. The appointment whic funded by the Science Research Cou will be for 3 years and the success applicant will be expected to take up hi her duties as soon as conveniently possi Salary on scale 1A £4,333 to £5,488 pa.

Applications, giving the names of t referees, should be sent to Dr D.R. Garr CRC Medical Oncology Unit, Cen Block, Southampton General Hospi Southampton SO9 4XY.

Please quote Ref: N. 3985(A

UNIVERSITY COLLEGE DUBLIN

POST IN MASS SPECTROMETRY

Applications are invited from suita qualified persons for a full-ti appointment in mass spectrometry. T post will involve the operation of a 70-70H Mass Spectrometer and Incos 2 Data System, providing a service teaching and postgraduate work within University. Candidates, preferal possessing an Honours degree equivalent qualification in Chemist Biochemistry, should have experience mass spectrometry and/or gas chron tography.

Salary £4,268 to £6,128 per annu Entry point may be above the minimum

Applications, together with the names two referees, should be forwarded to: T Secretary, Department of Agricultu Chemistry & Soil Science, Agricult Building, UCD, Belfield, Dublin 4.

The closing date for receipt applications is Friday, 28th Decemb 1979. 3964(A)

THAMES POLYTECHNIC

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH ASSISTANT IN PLANT BIOCHEMISTRY/ CELL BIOLOGY

Applications are invited from go honours graduates in biochemistry or related discipline to investigate changes the metabolism of polyamines and relat amino acids during the transition embryogenesis in cultured carrot cells, part of an on-going study into biochemic aspects of this transition. The work will jointly supervised by Dr A.W. Davids and Dr R.J. Yon. The successful applica will be expected to register for a high degree of the CNAA.

Salary scale: £3321 to £3705 inclusive.

Further particulars and form application may be obtained from t Staffing Officer, Thames Polytechn Wellington Street, London, SE18 6PF, whom completed applications should returned by 8th January 1980. 3969(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to

4 LITTLE ESSEX STREET, LONDON WC2R 3LF

TELEPHONE: 01 240 1101

Opportunities in Biological Development

G. D. Searle, whose modern, well-equipped research laboratories are located in pleasant surroundings at High Wycombe in Buckinghamshire, is currently engaged on exciting work in the field of Biological Development, and has created the following new positions:

Cell Biologist (Ref. CB/R15)

We require a PhD in animal cell physiology or related discipline to lead a small team in the development of scale up tissue culture systems, cell growth monitoring, and in the development of control systems for process optimisation.

The ideal candidate will have experience in animal cell culture, especially large scale culture, and may have experience in the manipulation of the synthesis of cellular synthetic processes.

Protein Chemist (Ref. PC/R15)

We require a PhD in protein chemistry or related field to provide new impetus and direction in this area and to play a central role in the protein purification group.

Applicants should have experience in protein isolation techniques and in analysis of complex protein mixtures. They must possess understanding of modern techniques to resolve interacting protein species, and knowledge of the factors contributing to protein stability in such systems.

Bioengineer (Ref. B/R15)

We require a Bioengineer to become involved in plant design, construction, operation and maintenance, and to develop process control systems.

Applicants should have a qualification of HND or degree standard and should have at least two years' experience in industry. A sound practical and theoretical knowledge of fermentation plant and equipment is essential. Experience with mammalian cell culture would be an advantage, and a working knowledge of electronics as applied to fermentations would be useful.

All these positions offer attractive salaries, and benefits include 4 weeks' holiday per year, contributory pension and private health insurance schemes, subsidized cafeteria, and sports and social club.

Please apply in the first instance to Miss D. Wardman, Assistant Personnel Officer, Searle Research & Development, Lane End Road, High Wycombe, Bucks. (Tel. High Wycombe 21124, ext. 3374).

SEARLE

3979(A)

UY'S HOSPITAL MEDICAL AND DENTAL SCHOOLS

e- or post-doctoral Research Assistant iochemist or Immunologist), with perience in biochemistry or immuno- emistry of bacteria, required for one year work on research project concerned with paration of specific components, pecially proteins from Streptococci. lary according to qualifications, with perannuation.

Apply in writing, with full curriculum tae, to the Secretary, Guy's Hospital edical School, London Bridge, SE1 9RT, oting Ref. IM7. 3970(A)

UNIVERSITY OF MASSACHUSETTS

DEPARTMENT OF BIOCHEMISTRY

OSTDOCTORAL POSITION

vailable immediately. Involving the plication of photoaffinity labelling chniques to the delineation of the pography of *E. coli* ribosome binding es for initiation factors. Research may so entail the application of other physico- emical techniques to probe the IF-3 nding site and mapping of the binding es of other factors (S-1, IF-2, EFTu).

Experience in protein synthesis/protein d nucleic acid chemistry is desirable. ppointment can be arranged for up to 2 ars. Salary range: $11-12,00.

Send resume and names of three ferences to: Dr Ira S Schwartz, epartment of Biochemistry, University of assachusetts, Amherst, MA 01003. An ual Opportunity/Affirmative Action mployer. W641(A)

ST THOMAS'S HOSPITAL MEDICAL SCHOOL (University of London)

JUNIOR/MEDICAL LABORATORY SCIENTIFIC OFFICER

required in the Endocrine laboratories to work on the development of methods used in the detection of anabolic steroids in athletes. Minimum qualification suitable A levels. Experience of Radioimmunoassay would be an advantage.

Salary in range £3,218 to £5,373 p.a. Applications to Professor R V Brooks, Department of Chemical Pathology, St Thomas's Hospital Medical School, London SE1 7EH. 3988(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

SENIOR LECTURER (Hon. Principal Grade)

physicist required to continue Radiotherapy Physics service and carry out development/research work and teaching in that subject. EMI-plan and Cathetron being installed.

Salary on Senior Lecturer Scale £8,698 to £10,775 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 11 January 1980. 3976(A)

NEW YEAR CLOSING DATES

In view of the New Year holiday Nature Classified will close as follows:—

Jan 3 issue closing
1 pm 27.12.79.

We are looking for a

RESEARCH TECHNICIAN

to work in a biochemical laboratory in Karlsruhe, Germany. The laboratory is located in a biology unit within the German nuclear research center. The site is 30 miles south of Heidelberg. The work involves cell culturing, microbiological and biochemical methods, gene cloning procedures. Salary is from 1800 to 2000 DM per month. Interviews could be in London or Karlsruhe.

Write before December 20 to:
Dr. B. Groner and Dr. P. Herrlich,
Kernforschungszentrum Karlsruhe,
IGT, Postfach 3640, 7500 Karlsruhe 1.

W632(A)

Kernforschungszentrum Karlsruhe GmbH Postfach 3640, 7500 Karlsruhe 1

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to

4 LITTLE ESSEX STREET, LONDON WC2R 3LF

TELEPHONE: 01 240 1101

ICI

CHEMIST/PHYSICIST
TO LEAD RESEARCH ON NMR TECHNIQUES

The ICI Corporate Laboratory, situated in Runcorn, Cheshire, is looking for an enterprising and imaginative PhD scientist (with or without post-doctoral experience) to take a leading role in developing the Company's use of advanced nuclear magnetic resonance techniques. He/she must have first class research experience in developing and/or applying NMR techniques in chemistry or biochemistry, and be keen to develop and exploit novel NMR applications in the chemical industry.

The Company's wide-ranging research interests will offer the successful applicant scope for working in a variety of areas, which may include organic and inorganic chemistry, catalysis, colloid science, materials science, biochemistry and biology. The direction of the work will depend to a large extent on his/her experience and research interests. An interest in novel biological applications would be an advantage, but applicants with other interests relevant to the industry's long-term technological needs will be considered.

In adddition to an attractive salary, there is a profit-sharing scheme and assistance with removal expenses.

If you are interested in this vacancy, please reply enclosing a full curriculum vitae to:—

Miss S E Bird, Personnel Officer
IMPERIAL CHEMICAL INDUSTRIES LIMITED
Corporate Laboratory
PO Box 11, The Heath, RUNCORN, Cheshire WA7 4QE

3967(A)

Physiologist/ Biochemist

We are seeking a Physiologist/Biochemist within our Toiletries Division to explore the biochemical and physiological situation of the mouth. The post which will be at Scientist level may, therefore, be expected to involve both laboratory benchwork and studies on human subjects. A wide knowledge of biochemical techniques will be necessary, so the successful candidate will probably possess relevant postgraduate experience, but equally important will be a pleasant personality with an ability to communicate on an abstract level. An interest in human psychology is essential.

The Company operates a progressive salary and promotion policy, good superannuation and life cover benefits are provided and, where appropriate, will assist with removal expenses.

The Laboratory is situated on the Wirral Peninsula, a few miles from the sea and convenient for Chester, Liverpool and North Wales.

Applications are invited from both males and females, who should write for an application form, quoting reference no. 665 M to

Personnel Officer, Unilever Research Port Sunlight Laboratory, Port Sunlight, Wirral, Merseyside, L62 4XN.
Closing date: 31 December, 1979. 3982(A)

unilever research

THE UNIVERSITY OF LANCASTER
DEPARTMENT OF ENVIRONMENTAL SCIENCES
RESEARCH ASSISTANT

Applications are invited for a Research Assistantship to work with Dr J Hamilton-Taylor and members of the Freshwater Biological Association on the investigation of heavy metal cycling in Windermere, Cumbria.

Applicants should be graduates in chemistry, geology or a biological/environmental science and have post-graduate/doctoral experience in environmental chemistry. The appointment, funded by NERC, will be for a period of three years and will commence as soon as possible. The starting salary will be in the range £3775 to £4333 depending on qualifications.

Further particulars may be obtained (quoting reference L173) from the Establishment Officer, University House, Bailrigg, Lancaster, LA1 4YW, to whom applications (five copies), naming three referees, should be sent by 14 January, 1980. 3968(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF BACTERIOLOGY
LECTURER

Applications are invited for the post of Lecturer with teaching and research duties in laboratory aspects of infectious disease. Candidates should have a medical or higher science qualification, with experience in medical laboratory microbiology and undergraduate teaching and preferably with an interest in clinical bacteriological research and development.

The clinical salary scale for medically qualified lecturers is £5,316 to £9,420. The non-clinical scale for science graduates is £4,333 to £8,992. USS Superannuation.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, with whom applications (8 copies) including curriculum vitae and the names and addresses of two referees should be lodged not later than 20th January, 1980. Please quote Reference 1090. 3983(A)

Please mention
Nature
when replying to these advertisements

UNIVERSITY OF STIRLING
DEPARTMENT OF BIO-CHEMISTRY
GRADE III RESEARCH TECHNICIAN

Applications are invited for the above post to work on a 3 year project funded by the Science Research Council to study the process of refolding of multisubunit enzymes. Candidates should possess a degree or equivalent in Bio-chemistry or a related subject and preferably some relevant experience. The starting salary is up to £3291 rising by 2 increments to £3468. It may be possible for the successful applicant to register for a higher degree.

Application forms obtained from the Secretary, University of Stirling, Stirling FK9 4LA. Tel: Stirling 3171 ext 2314 to be completed and returned by 31st January 1980. 3965(A)

THE UNIVERSITY OF GENEVA MEDICAL SCHOOL
has an opening for a position of
FULL PROFESSOR OF BIOCHEMISTRY

Send applications with curriculum vitae description of previous experience and activities, list of publications, to Prof. B Mach, Chairman of Search committee Faculté de Médecine, Quai Ernest Ansermet 20, 1211 Geneva 4, Switzerland until February 29, 1980. W640(A)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF VETERINARY PHYSIOLOGY
POSTDOCTORAL RESEARCH FELLOW

Required as soon as possible to work on excitation-secretion coupling in insect salivary glands with Dr C.R. House.

Electrophysiological experience is desirable. The post is tenable until 30/6/81, and the salary is on Range 1A of the scales for Research and Analogous Staff (plus superannuation).

Applications, by letter (two copies), giving the names of two referees, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, not later than January 12th 1980. Please quote reference 5070. 3966(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
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TELEPHONE: 01 240 1101

ALLIED MEDICAL GROUP
Kingdom of Saudi Arabia
The Riyadh Al-Kharj Hospital Programme
MEDICAL LABORATORY SCIENTIFIC OFFICERS

Excellent new opportunities have now arisen for Medical Laboratory Scientific Officers at this hospital programme providing high quality health care to the Armed Forces of Saudi Arabia. Posts will be based at the 314 bed Riyadh Military Hospital and include rotational duties at the 50 bed Al-Kharj Hospital, 80 km to the South East of Riyadh.

MLSO's in the following specialities are required:

Blood transfusion/Haematology	Ref. RKH10
Microbiology	Ref. RKH11
Biochemistry	Ref. RKH12

Minimum qualifications are HNC, with 3 years experience.

SALARY 59,800 Saudi Riyals per annum (Saudi Riyals approx 7.4 to £)

Broad Terms and Conditions

Contracts: Appointments will be made for two years initially and renewable by mutual consent.

Taxation: There is no income tax in the Kingdom of Saudi Arabia and no restrictions on exchange/transfer of currency.

Gratuity: A gratuity of one months salary for each completed year of service will be payable on completion of the contract.

Travel and Leave: Free air fares and four weeks leave after completion of each 26 weeks satisfactory service.

Accommodation: Free accommodation, Recreation, Welfare and Transport.

Medical Care: Medical Care for Officers.

Method of Application

If you are interested in this challenging opportunity, please write or telephone for an application form and further details quoting the reference number to:

David Campbell
Manager, Personnel Services
Allied Medical Group Limited
18 Grosvenor Gardens
London SW1W 0DZ
Tel: 01 730 4511 Ex. 293

Closing date 10 days from date of publication.

3922(A)

Allied Medical Group

UNIVERSITY OF GLASGOW

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work with Drs P S Braterman and A G Cairns-Smith on a two-year study, supported by NERC, of photoinduced redox reactions in simulated Precambrian seas. Experience in electrochemistry or photochemistry would be an advantage.

Salary will be on Range 1A of the salary scales for Research and Analogous Staff (£4,333 to £4,622, under review) plus USS.

Applications, including the names of two referees, should be sent to Dr A G Cairns-Smith, Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ, from whom further particulars are available.

In reply please quote Ref. No. 4561M.

3994(A)

NUFFIELD LABORATORIES OF COMPARATIVE MEDICINE
Zoological Society of London
RESEARCH ASSISTANT

with First Class Degree in Nutrition and/or BIO-CHEMISTRY required for 3 year project to study Nutrition in the management of Multiple Sclerosis involving the analysis of food intakes of patients and use of laboratory methods to study the relevance of diet to the metabolism of the central nervous and immune systems. Experience of lipid and prostaglandin assays useful.

Salary in the range £5,058 to £6,504 according to age and experience with USS/USDPS benefits. Apply in writing to Assistant Establishment Officer, Zoological Society of London, Regent's Park, London, NW1 4RY.

3995(A)

ANNOUNCEMENTS

A NATO Advanced Study Institute on Chemically Sensitive Electronic Devices will be held at the Peddie School, Hightstown, NJ from 9 June to 21 June 1980. The fundamental principles of transducer action for chemical sensors, the resulting device physics and selected areas of application will be presented in a series of lectures by: P Bergveld and G A Bootsma, Twente Technical University; R Buck, University of North Carolina; G Farrington, General Electric; M Green, Imperial College; T. Matsuo of Tohoku University; I Lundström of Linköping University; I Lauks and J N Zemel, University of Pennsylvania; S Pace, Stauffer Chemical.

This course is primarily intended for the young researcher at the post doctoral level but consideration will be given to anyone in the process of initiating research in these areas. The student body will be sought from a broad range of disciplines such as: electrical engineering, chemistry and chemical engineering, physics, material science and biomedical science and engineering. Limited travel and living assistance is available. Special arrangements are being made to provide low cost travel to and from the Peddie School.

For further information and applications write to either:

J N Zemel
University of Pennsylvania,
Moore School/D2,
200 S. 33rd Street,
Philadelphia, Pa. 19104. USA.

P Bergveld
Twente University of Tech.
Dept. of Electrical Eng'g.
P.O. Box 217,
Enschede, Netherlands.

W639(G)

ASSISTANTSHIPS

UNIVERSITY COLLEGE CARDIFF

Applications are invited for two

POST-DOCTORAL RESEARCH ASSISTANTSHIPS in the

DEPARTMENT OF BIOCHEMISTRY

(a) The study and isolation of enzymes responsible for alcohol and wax-ester synthesis in bacteria, and their role in the regulation of membrane function (with Dr. N J Russell). The project will involve the purification and characterisation of membranes and individual enzymes, radio-chemical enzyme assays and the setting-up of capillary-g.l.c. equipment.

(b) A study of the distribution and metabolism of chloroplast thylakoid lipids (with Dr J L Harwood). This project will involve the isolation and characterisation of sub-cellular particles, examination of lipid sidedness by chemical and enzymatic techniques and a study of the turnover of individual components *in vivo*.

Both positions are funded by the S.R.C. for three years. Initial appointment will be at the 1st-3rd points of the 1A scale (£4333 to £4910, under review) according to age and experience.

Applications (2 copies) including a curriculum vitae together with the names and addresses of two referees and indicating the post of interest, should be sent to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL. Closing date 31st January 1980. Reference 1946. 3980(P)

WORKSHOP

TEPHRA STUDIES AS A TOOL IN QUATERNARY RESEARCH

A NATO Advanced Studies Institute on this subject will be held in Iceland from June 18th to 30th 1980. The ASI will consider all applications of tephra (volcanic ash) studies to Quaternary geological problems: dating, correlation, stratigraphy on land and in the ocean, history of active volcanoes, eruption prediction and styles of volcanism. The ASI will include 5 days of field excursions in Southern Iceland examining pyroclastic deposits. Participants from NATO countries are eligible for partial financial support. Interested persons should apply for further information to:—

Dr R S J Sparks, Department of Mineralogy and Petrology, University of Cambridge, Cambridge CB2 3EW, England. 3981(V)

SCHOLARSHIPS

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHYSICS

Applications are invited from United Kingdom citizens holding a good Honours degree in physics, chemistry, mechanical engineering or similar discipline for a

RESEARCH SCHOLARSHIP

tenable for three years from February 1980 for a PhD project on mechanical memory metals.

The scholarship is funded by the British Gas Corporation at a rate somewhat greater than the normal SRC research studentship.

Applicants should write as soon as possible, naming two referees, to Dr K H G Ashbee, H H Wills Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL.

3989(H)

COURSES

Under the sponsorship of the European Communities, the Department of Molecular Biology and Plant Physiology, University of Aarhus, Denmark, is organizing an interdisciplinary laboratory course to take place in Aarhus, from 17 to 30 August 1980 on

Organization and function of Specific Eukaryotic Genes

Organizers: Ole Westergaard, Johan C. Leer and Ole F. Nielsen

Participants in the course will perform a variety of fundamental experiments on the molecular biology of eukaryotic genes with emphasis on chromatin: Mapping of coding and intervening sequences on specific genes; chromatin structure of active and inactive genes; transcription of specific genes; replication and repair on the level of chromatin. Seminars and discussions will complete the practical work.

The teaching staff will include:

Christine Brack, Basel
Richard Braun, Bern
Sergey Borchsenius, Leningrad
Gunna Christiansen, Aarhus
James E. Cleaver, San Francisco
Bertil Daneholt, Stockholm
Sarah Elgin, Cambridge, USA
Jan Engberg, Copenhagen
Richard Flavell, London
Hans Klenow, Copenhagen
Ron Laskey, Cambridge
Alan R. Lehman, Brighton
Pierre Oudet, Strasbourg
Lennart Philipsson, Uppsala
Rudi Planta, Amsterdam
Robert Roeder, St. Louis
Michael Trendelenburg, Heidelberg

Staff of Department of Molecular Biology and Plant Physiology, University of Aarhus.

The number of participants will be limited.

The working language of the course will be English.

Applications for enrolment should be sent before 20 March 1980 to the Molecular Biology and Radiobiology Course Secretariat, Commission of the European Communities, DG XII, 200 rue de la Loi, B-1049 Brussels and should contain the following items: (1) name, address and academic affiliation; (2) age and nationality; (3) scientific curriculum; (4) statement on knowledge of languages; (5) list of publications, if any; (6) motivation for the application; (7) a letter from the applicants head of laboratory; (8) any other information that may be useful to the selection committee in evaluating the application. Further information may be obtained from the same address. W631(D)

The Advertisement offices for NATURE have moved to
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
TELEPHONE: 01-240 1101

MOLECULAR AND CELLULAR ASPECTS OF EARLY MAMMALIAN DEVELOPMENT ADVANCED STUDY COURSE: CRETE, SEPTEMBER 21st-28th 1980

Applications are invited from suitably qualified research workers for attendance at this lecture and discussion group course on modern approaches to the study of early mammalian development co-sponsored by The European Molecular Biology Organization and The Rockefeller Foundation. The Organizing Committee of W.D. Billington (Bristol), M.I. Sherman (New Jersey), A. Psychoyos (Paris), A. McLaren (London) and J. Danezis (Athens) will be assisted in the teaching by a panel of distinguished international experts in this field. Instruction will be entirely in English. The course will be held on the island of Crete immediately following the International Embryological Conference in Athens and its location in a small Academy will allow teachers and participants to be accommodated together in an informal atmosphere conducive to the fullest exchange of information and ideas in this important and rapidly expanding subject.

There will be a registration fee of US$60 but accommodation, meals and transport from Athens — Crete will be provided free of charge. Some bursaries to help towards travel expenses may also be available for applicants with limited financial resources. Attendance will be limited to 80-100 participants, mainly in the early post-doctoral stages of their career, and early application is advised. **Further details and forms of application are available from Dr. W.D. Billington, Department of Pathology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD, UK, to whom completed applications must be returned not later than March 31st 1980.** 3977(D)

FELLOWSHIPS

UNIVERSITY OF OXFORD
BRASENOSE COLLEGE
PLATNAUER JUNIOR RESEARCH FELLOWSHIP

The College proposes to elect a Platnauer Junior Research Fellow (male or female) in the Natural Sciences or Mathematics.

The tenure will be for three years from 1 October 1980 with the possibility of renewal for a further three years. The stipend of a Junior Research Fellow is at present £3,200 per annum and is subject to periodical review. Other benefits include free accommodation, or an allowance in lieu, and a grant for approved research expenses, which may include travel.

There will be no examination. Election will be made after interview.

Candidates must, within the seven years ending 1 October 1980, have either qualified for the degree of BA at Oxford or obtained an equivalent qualification elsewhere.

Applications accompanied by a curriculum vitae and the names of three referees should reach the Principal of Brasenose College, from whom further information may be obtained, not later than 18 January 1980. 3978(E)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF PATHOLOGY
RESEARCH FELLOWSHIP

Applications are invited for a post-doctoral Research Fellowship in a group studying the factors responsible for DNA stability and degradation within mammalian cells throughout the cell cycle and during programmed cell death. Experience with mammalian or viral chromatin is desirable but candidates with a relevant cell biological background will be viewed favourably. The post is available now and is supported by the Cancer Research Campaign on the nationally agreed 1A Salary Scale for 3 years in the first instance with a possibility of further renewal.

Applications enclosing a Curriculum Vitae and the names of two referees should be sent to Dr A H Wyllie, Department of Pathology, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG, by 25th January, 1980. Please quote Reference 5074 3991(E)

INTERNATIONAL INTERNSHIPS AND RESEARCH FELLOWSHIPS at the INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

A limited number of research Internships and Fellowships are available within multi-disciplinary research programs for the rainfed semi-arid tropics. Potential research areas: plant breeding, genetics, physiology, pathology, entomology, biochemistry, microbiology, agroclimatology, agronomy, cropping systems, soil science, land and water management, agricultural engineering.

Requirements: Recent PhD or MSc, research interest within ICRISAT mandates. Competitive compensation, furnished apartment or dormitory, economy class air fares. Apply to Director, ICRISAT, Patancheru PO, AP 502 324, India. W635(E)

nature

NEW YEAR CLOSING DATES

In view of the New Year holiday Nature Classified will close as follows:—

Jan 3 issue closing
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Call toll-free: 800-225-1572
(In Massachusetts and International: 617-482-9595)
NEN Chemicals GmbH, Dreieich, W. Germany. NEN Canada Ltd., Lachine, Quebec

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NEW PRODUCTS RESTRICTION ENDONUCLEASES

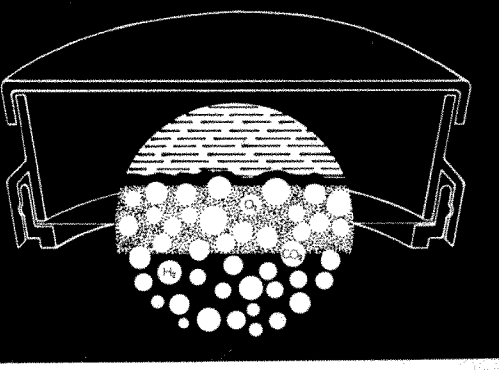
RESTRICTION ENDONUCLEASE	PRICE	RESTRICTION ENDONUCLEASE	PRICE
Alu I (Cat. No. EEN-201)	$ 50.00/200 units $205.00/1000 units	Hind III (Cat. No. EEN-210)	$ 75.00/1000 units $275.00/5000 units
Bam HI (Cat. No. EEN-202)	$ 50.00/1000 units $205.00/5000 units	Hha I (Cat. No. EEN-213)	$ 50.00/1000 units $205.00/5000 units
Bgl I (Cat. No. EEN-203)	$ 50.00/100 units $205.00/500 units	Hpa I (Cat. No. EEN-215)	$ 75.00/100 units $275.00/500 units
Bgl II (Cat. No. EEN-204)	$ 75.00/100 units $275.00/500 units	Hpa II (Cat. No. EEN-216)	$ 50.00/500 units $205.00/2500 units
Eco RI (Cat. No. EEN-205)	$ 75.00/1000 units $275.00/5000 units	Mbo I (Cat. No. EEN-218)	$ 50.00/1000 units $205.00/5000 units
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Hae III (Cat. No. EEN-208)	$ 50.00/1000 units $205.00/5000 units	Pvu I (Cat. No. EEN-224)	$ 50.00/100 units $205.00/500 units
Hinc II (Cat. No. EEN-209)	$ 75.00/200 units $275.00/1000 units	Pvu II (Cat. No. EEN-225)	$ 95.00/500 units $195.00/1000 units
Hind II (Cat. No. EEN-223)	$ 50.00/500 units		

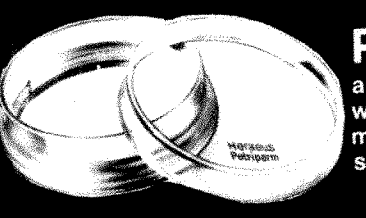
For more information: Write or Call

ENZO BIOCHEM, INC.
300 Park Avenue South
New York, N.Y. 10010
(212) 533 4940

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A new approach in tissue culture



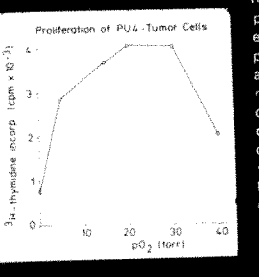


PETRIPERM
a novel culture dish with gas permeable membrane as cellular support.

Additional advantages of the PETRIPERM membrane are:

- UV permeable 200 nm
- Suitable for high power light and fluorescence microscopy
- The membrane can be cut for cell cloning, electron microscopy, staining and documentation
- Chemically resistant to acids, bases and organic solvents

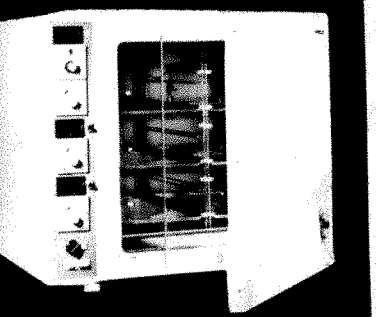
PETRIPERM is available either with a hydrophilic surface for optimal attachment, or with a hydrophobic surface for easy detachment of adherent cells.



In the intact animal, the pH, and the partial pressure of both oxygen and CO_2 of the cell environment are under total biochemical and physiological control. This state is not even approximated by available techniques of monolayer cell culture in conventional tissue culture ware. Continuously changing gradients of these parameters in the micro-environment of cultured cells will obscure the biological significance of culture dependent phenomena. PETRIPERM is the prerequisite for the precise control of pO_2, pCO_2 and pH in the cellular environment as gases reach the cultured cells directly through the highly gas permeable 75 μm thick membrane.

Heraeus— Co_2-Incubator B 5060 EK/CO_2

We build the incubator with appropriate pCO_2, pH and pO_2 control units



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The LEITZ DIALUX 20 has been designed with an eye to the future development of microscopy: the established Leitz modular principle has been extended as a result of current experience: it is now even more practical, and offers optimum operating convenience with maximum functional reliability.

A considerably improved arrangement of the optical illuminating system makes the LEITZ DIALUX 20 even more efficient for all optical examinations of transparent specimens. It has been possible to enlarge the microscope field of view even further through the use of the excellently corrected series of LEITZ NPL FLUOTAR objectives. Amounting to more than 23% when compared with that of conventional microscopes – with critical definition across the entire image, this ensures that the new LEITZ DIALUX 20 is not only a reliable tool for diagnostic microscopy, but also a basic instrument that has the necessary reserves for biological research. We should like to convince you of the extremely favourable relationship between performance and price.

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☐ I should like to arrange a date for the Leitz consultant to visit m

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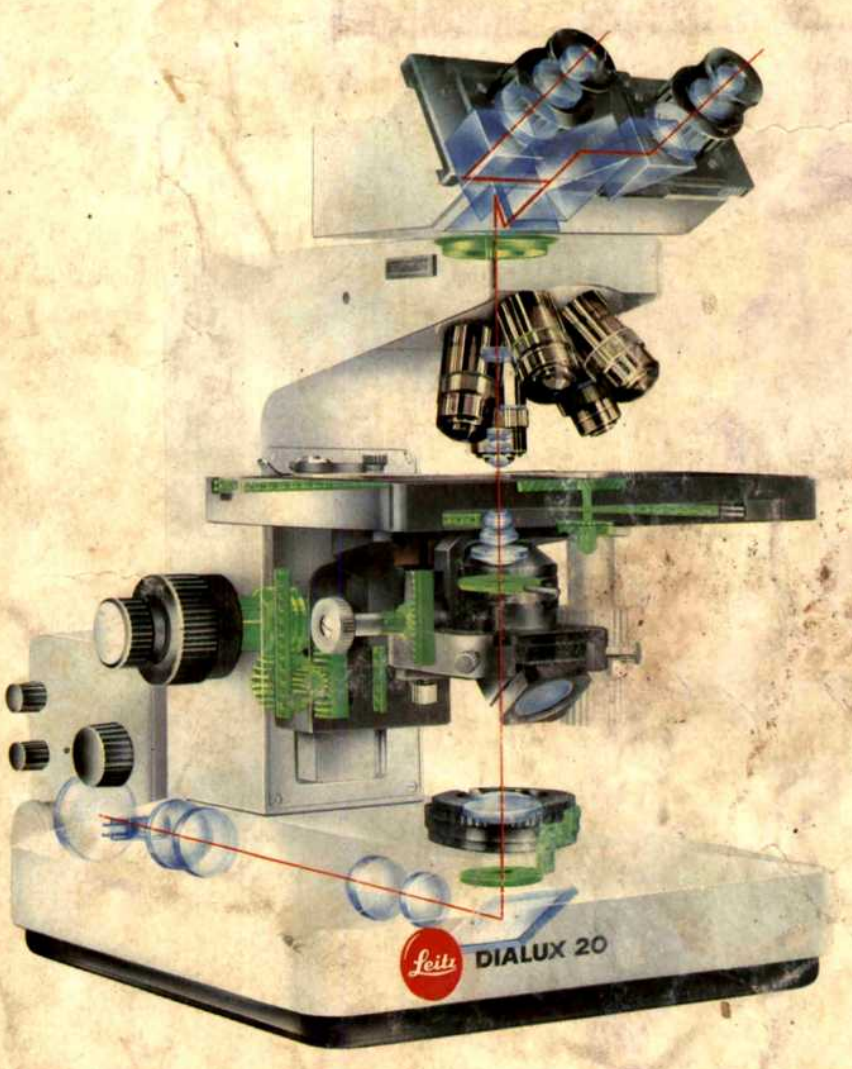
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Please return the coupon or write to: 409 Information Service, Ernst Leitz Wetzlar G.m.b.H., P.O.B. 2200, D-6330 Wetzlar

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